ENDOCRINOLOGY

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VOLUME 23
July-December, 1938

SURELY every medicine is an innovation; and he that will not apply new remedies, must expect new evils.—Francis Bacon, *Essaye of Innovations*

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Errata, Volume 23

On page 622 the seventh line from the bottom—"activity . . . conditioned" should be inserted between lines 3 and 4 of page 623.

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ENDOCRINOLOGY

THE BULLETIN OF
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VOLUME 23 JULY, 1938 NUMBER 1

THE INHIBITION OF UTERINE BLEEDING WITH ESTRADIOL AND PROGESTERONE AND ASSOCIATED ENDOMETRIAL MODIFICATIONS[1]

FREDERICK L. HISAW AND ROY O. GREEP
The Biological Laboratories, Harvard University
CAMBRIDGE, MASSACHUSETTS

The parts played by the follicular and corpus luteum hormones in the development of the endometrium are quite well understood, at least from a general qualitative standpoint. Allen (1) found that the normal phenomena associated with the follicular half of the menstrual cycle of monkeys could be reëstablished in castrated animals by daily injections of estrin. It is also known that the normal conditions found in the genital tract during the luteal half of the menstrual cycle can be duplicated in castrated animals by administering progestin (2, 3, 4). The ability of these hormones to inhibit uterine bleeding seems equally well established. Allen (1) demonstrated that estrin would inhibit the postoperative bleeding which usually follows total castration, whereas Hisaw (3) found that the dosage of estrin required to postpone such bleeding depended upon the time during the menstrual cycle at which the ovaries were removed. When castration was performed previous to ovulation, relatively small doses of estrin were effective, but as the endometrium was being transformed into a premenstrual condition, it became progressively more difficult to inhibit bleeding. Engle et al (4) have also shown that it is extremely difficult, if not impossible, to prevent involution of the endometrium and consequently bleeding by giving estrin after a premenstrual condition has been established by a series of injections of progestin. In contrast with the apparently limited ability of estrin to prevent bleeding, Smith and Engle (5), Hisaw (3) and Engle et al (4) have shown that progestin will inhibit bleeding from an endometrium of either an estrin or progestin type for an indefinite period.

It has been recently shown by Hisaw et al (6) that in the development of the endometrium a synergism exists between estrin and progestin, in that the two hormones seem more effective when given simultaneously than when

[1] Aided by a grant from the Rockefeller Foundation.

TABLE 1

Animal	Previous Treatment	Days
A1.55	100 RU 19	25 RU 6
A1.62	100 RU 21	50 RU 9
A1.65	100 RU 21	50 RU 9
A1.66	100 RU 21	50 RU 10
A1.56	100 RU 21	¼ RbU 6
A1.53	MS	½ RbU 8 (1)
A1.56	100 RU 20	½ RbU 8 (2)
A1.57	AP	½ RbU 8 Killed
A1.53	100 RU 23	½ RbU 11 (2)
A1.58	AP	½ RbU 13
A1.56	100 RU 20	¾ RbU 9
A1.61	AP	¾ RbU 15
A1.60	AP	¾ RbU 19 Killed
A1.53	100 RU 20	¾ RbU 19
A1.55	AP	¾ RbU 24
A1.59	100 RU 16	¾ RbU 25
A1.56	100 RU 20	1 RbU 9
A1.58	100 RU 21	1 RbU 27
A1.53	100 RU 19	1 RbU 31 Killed
A1.55	100 RU 19	1 RbU 44 ½ RbU 4
A1.52	100 RU 19	1 RbU 44 ½ RbU 7
A1.66	100 RU 20	25 RU + ¼ RbU 8
A1.67	100 RU 20	25 RU + ¼ RbU 10
A1.65	100 RU 20	25 RU + ¼ RbU *10 12
A1.55	100 RU 20	25 RU + ¼ RbU 14
A1.65	100 RU 20	25 RU + ¼ RbU 14
A1.58	100 RU 21	25 RU + ½ RbU 9
A1.67	100 RU 21	25 RU + ½ RbU *21 24
A1.62	100 RU 21	25 RU + ½ RbU 18 500 RU 18 100 RU + 1 RbU * 10 500 RU 3 (5)
A1.56	100 RU 20	25 RU + ½ RbU 19
A1.52	100 RU 20	25 RU + ½ RbU 21 500 RU 10 100 RU + 1 RbU 10 500 RU 3
A1.52	100 RU 21	50 RU + ¼ RbU 13
A1.68	100 RU 21	50 RU + ¼ RbU *21 28 50 RU 4
A1.52	AP	50 RU + ½ RbU 19 (4)
A1.56	100 RU 33	50 RU + ½ RbU 21 50 RU 3
A1.58	100 RU 33	50 RU + ½ RbU * 22 100 RU 2
A1.52	100 RU 33	50 RU + ½ RbU 22 500 RU 4
A1.55	100 RU 20	50 RU + ½ RbU 20 500 RU * 10 (3) 50 RU + ½ RbU 7

AP Follicular development induced by pituitary extracts previous to castration and injection of progesterone.

MS Given pregnant mare serum previous to castration and injection of progesterone.

(1) Given oestrin-free progestin. All others received synthetic progesterone.

(2) Given 100 RU and 50 RU of oestrin the first two days of progesterone treatment.

(3) Given 250 RU and 100 RU of oestrin the first two days of progesterone treatment.

(4) Threads placed through uterus on the ninth day of treatment.

(5) Bleeding started on the second day.

* Uterine biopsy.

separated into two series of injections. Unpurified extracts were used in most of these experiments, so that an exact quantitative estimate of the dosage was not possible. The present study is an attempt to determine the quantitative relationship of chemically pure estradiol and progesterone for the inhibition of bleeding following an estrin treatment, and for the development of a premenstrual endometrium.

PROCEDURE

The general procedure followed was to give a preliminary treatment of 100 R.U. (Allen-Doisy) of estrin daily for about 20 days to castrated adolescent monkeys, followed by injections of chemically pure estradiol, progesterone, or combinations of the two hormones.[2] The estradiol and progesterone were dissolved in sesame oil and injected subcutaneously. The progesterone was injected twice daily and estradiol once each day, and when both hormones were used they were given separately. The success or failure of a given treatment to inhibit bleeding was measured by the length of time between the discontinuance of the preliminary injections of estrin and the appearance of macroscopic amounts of blood in the vaginal lavage. Modifications of the endometrium were followed by taking uterine biopsies and by killing animals at selected times.

EXPERIMENTAL RESULTS

The inhibition of uterine bleeding. It has been known since the early experiments by Allen (1) that uterine bleeding almost invariably occurs after injections of estrin are discontinued. It can be stated with a great degree of certainty that the animals in the present experiments (table 1) would have bled within a very few days after cessation of the preliminary estrin treatment. It is also equally certain that the endometria were comparable in that each must have shown the typical morphological characteristic of the action of estrin at the time the experiments in point were started.

When 100 R.U. of estrin are given daily for approximately 20 days and the treatment stopped, bleeding may be expected to begin about 4 to 6 days later. On this basis, it is seen in table 1 that 25 R.U. daily had little if any effect, whereas 50 R.U. showed a definite inhibitory action as bleeding was postponed until the 9th to 10th day. Likewise ¼ RB.U. of progesterone was ineffective, whereas higher dosages gave unquestionable inhibition. Bleeding started between the 8th and 13th days when ½ RB.U. was given daily, between the 15th and 25th when ¾ RB.U. was injected, and one animal bled on the 27th day, another was not bleeding when killed on the 31st, while 2 others had not bled at the 44th day when given 1 RB.U. daily. One exception, however, must be made to this statement. It should be noted that monkey *A1.56*[3] bled on the ninth day in 2 experiments when given either ¾ RB.U. or 1 RB.U. of progesterone.

[2] The estrone used in the 'priming' treatment for most of the animals and in a number of experiments was injected either alone or in combination with progesterone for the inhibition of bleeding. We wish to thank Dr. J. A. Morrell, E. R. Squibb and Sons for this material. The estradiol and progesterone were furnished by Dr. Erwin Schwenk, Schering Corporation, for which we are very grateful. Estrone and estradiol were dissolved in oil and standardized by the method of Allen and Doisy. One R.U. of estradiol, as used here, is equivalent to 1/12γ, and 1 R.U. of estrone is equal to 3 I.U. One RB.U. of progesterone is equivalent to 1 mg. of the synthetic compound. No physiological difference was discovered between the action of estrone and estradiol when used on the same rat unit basis.

[3] Throughout this paper "*A1*" has the same significance as "*Al*" in related papers from this laboratory.

A general conclusion which may be drawn from these results is that both estrin and progesterone possess the ability to inhibit uterine bleeding under the conditions set forth in these experiments, and that their inhibitory power is correlated with the amount of hormone injected. With regard to progesterone, a daily dosage of 1 Rb.U. is the minimum required to postpone bleeding indefinitely.

An additional point of interest is the fact that when given 1 Rb.U. of progesterone daily *A1.55* and *A1.52* had not bled at the end of 44 days; but bleeding started 4 and 7 days respectively after the dosage was reduced to ½ Rb.U. daily. When one considers the probable condition of such endometria on the 44th day, this seems all the more important, as can be judged by studying the endometrium of *A1.53,* which received similar treatment and was killed on the 31st day. The gross histology of the glands of this endometrium (fig. 1) does not suggest progesterone effects. In fact, it resembles that of untreated castrated animals although, as will be discussed later, there are certain important cytological differences. Nevertheless, the implication is that it was from an endometrium of this type that bleeding occurred in *A1.55* and *A1.52,* when the daily dosage of progesterone was reduced to ½ Rb.U.

When estradiol and progesterone were injected simultaneously, it was found that dosages of either hormone, which would not postpone bleeding when given alone, gave definite inhibition when administered together. Daily injections of either 25 R.U. of estradiol or ¼ Rb.U. of progesterone had no significant influence on bleeding, but when the two were given simultaneously bleeding did not occur until the 8th to the 14th day. Also, 50 R.U. plus ¼ Rb.U. inhibited bleeding for 13 days in one animal (*A1.52*) and bleeding had not occurred at the end of 28 days in another (*A1.68*). A combination of 25 R.U. and ½ Rb.U. postponed bleeding for 9, 19, and 24 days in 3 experiments, and in 2 others the animals showed no indications of bleeding on the 18th and 21st days, when the treatment was changed. When 50 R.U. plus ½ Rb.U. were injected daily into 5 animals, only one bled during the 19 to 22 days of treatment. This monkey (*A1.52*), however, had threads placed in the uterus on the ninth day of treatment, which may have been responsible for the bleeding.

These results show that ¼ Rb.U. of progesterone in combination with 50 R.U. of estradiol gives about the same degree of inhibition as ¾ Rb.U. of progesterone alone. Also a dosage of 25 R.U. plus ½ Rb.U. does not seem as effective as 50 R.U. plus ½ Rb.U. The combination of 50 R.U. plus ½ Rb.U. is approximately the minimal balance that will give consistent inhibition for an extended period, and in this regard is equivalent to 1 Rb.U. of progesterone.

In a few experiments in which bleeding had been successfully prevented for a considerable period by the simultaneous action of the two hormones, progesterone was withdrawn to see if the inhibition could be prolonged with estradiol alone, at the same or at an increased dosage. It will be seen (table 1) that 2 animals (*A1.56* and *A1.68*) receiving 50 R.U. plus ½ Rb.U. and 50 R.U. plus ¼ Rb.U. daily bled on the third and fourth day respectively after the progesterone injections were discontinued. Another (*A1.58*), under simi-

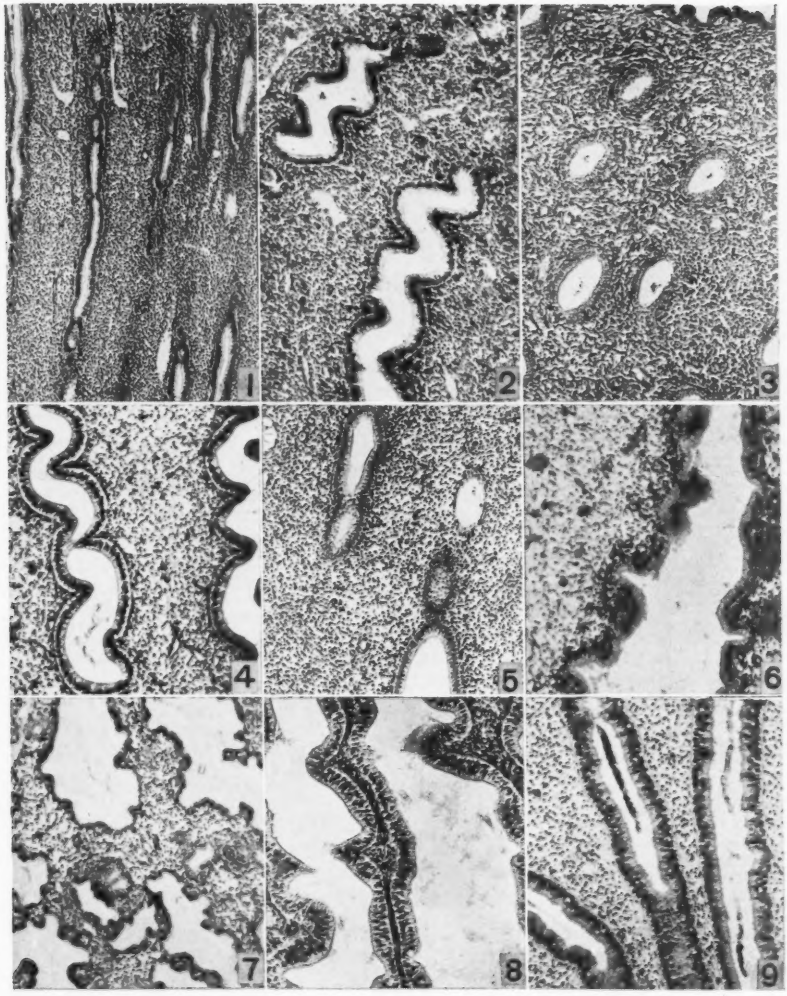

Fig. 1. Endometrium of monkey *A1.53*. Given 1 Rb.U. progesterone daily for 31 days. x80.
Fig. 2. Endometrium of monkey *A1.57*. Given ½ Rb.U. progesterone daily for 8 days. x80.
Fig. 3. Endometrium of monkey *A1.60*. Given ¾ Rb.U. progesterone daily for 19 days. x80.
Fig. 4. Uterine biopsy of monkey *A1.65*. Was given 25 R.U. estrin plus ¼ Rb.U. progesterone daily, biopsy taken on the 10th day, and bleeding began on the 12th. x80.
Fig. 5 Uterine biopsy of monkey *A1.68*. Received 50 R.U. plus ¼ Rb.U. daily; biopsy taken on the 21st day. x80.
Fig. 6. Uterine biopsy of monkey *A1.67*. Given 25 R.U. plus ½ Rb.U. daily; biopsy taken on the 21st day. x80.
Fig. 7. Uterine biopsy of monkey *A1.58*. Received 50 R.U. plus ½ Rb.U. daily; biopsy taken on the 22nd day. x80.
Fig. 8. Uterine biopsy of monkey *A1.62*. Received 25 R.U. plus ½ Rb.U. daily for 18 days, bleeding was inhibited for the succeeding 18 days by injecting 500 R.U. daily, and 100 R.U. plus 1 Rb.U. was given daily for an additional 10 days, at the conclusion of which the biopsy was taken. x80.
Fig. 9. Uterine biopsy of monkey *A1.55*. 50 R.U. plus ½ Rb.U. was injected daily for 20 days, bleeding was inhibited for an additional 10 days by giving 500 R.U. daily, at the conclusion of which the biopsy was taken. x80.

lar conditions, bled on the second day when the treatment was changed to 100 R.U. of estradiol daily, whereas inhibition was definitely maintained in 3 out of 4 experiments (*A1.62, A1.52, A1.55* and *A1.52*) when a daily dosage of 500 R.U. of estradiol was given.

The 3 animals (*A1.62, A1.52* and *A1.55*) in which bleeding had been successfully inhibited for 10 to 18 days after progesterone withdrawal by the injection of 500 R.U. of estradiol daily were used in experiments to test the possibility of further postponement of bleeding by a second treatment with a combination of the two hormones. In the first experiment (*A1.55*) 250 R.U. plus ½ Rb.U. were given the first day, 100 R.U. plus ½ Rb.U. the second, and 50 R.U. plus ½ Rb.U. daily thereafter. Bleeding started on the seventh day, indicating that the original combination of estradiol and progesterone which had prevented bleeding for 20 days would not do so after inhibition for 10 days with a daily dosage of 500 R.U. of estradiol. In the other two experiments, a dosage of 100 R.U. plus 1 Rb.U. was given for 10 days without bleeding, but menstruation started on the third day after progesterone was withdrawn, and estradiol increased to 500 R.U. daily.

These results are in general agreement with the observation that one of the effects of the corpus luteum hormone on the endometrium is to establish a condition that makes it more difficult for estrin to prevent menstruation after the withdrawal of progesterone. The fact that 500 R.U. of estradiol did inhibit bleeding when preceded by combinations containing ½ Rb.U. of progesterone and did not, after a dosage of 100 R.U. plus 1 Rb.U., suggests that there is a quantitative relationship between the extent of progesterone stimulation of the endometrium and the amount of estrin required to prevent bleeding. Although the observations are too few to permit a definite statement, a study of the endometria does indicate that the increased resistance to estradiol inhibition is not closely correlated with the histological condition of the glands and does suggest the possibility of vascular changes as the probable cause.

HISTOLOGY

The observation that estradiol and progesterone inhibit uterine bleeding more effectively when administered simultaneously than when injected separately in comparable doses raises the question as to whether there is a correspondingly greater histological modification of the endometrium when the two hormones are combined. This is of particular importance with regard to the ability of progesterone to produce premenstrual changes in the endometrium when given alone and in combination with estrin. Information regarding this was obtained by killing representative animals, and by taking endometrial biopsies during the course of the experiments.

The minimal daily dose of progesterone which gave definite inhibition of bleeding was ½ Rb.U., and monkey *A1.57* was used as a representative of this group. The uterus was removed on the eighth day of progesterone treatment, and the endometrium (fig. 2) showed degenerative changes which normally precede bleeding. The glands were only slightly dilated, moderately coiled, and a few showed the beginning of secretion, whereas the stroma was quite dense. The general condition indicated a mild progesterone stimulation.

Monkey *A1.60*, receiving ¾ Rb.U. of progesterone daily and killed on the 19th day, showed a more advanced premenstrual development of the endometrium than *A1.57*. The uterine glands of *A1.60* (fig. 3) were actively secreting throughout most of their distal parts in the compactum, whereas the glands of *A1.57* were mostly in a presecretory condition. The glandular epithelium decreased in thickness and the nuclei, as seen in sections, tended to be arranged in a single row close to the basement membrane. The endometrium seemed to be approaching a condition previously described as secretory exhaustion (3).[4]

The endometria of 2 monkeys, *A1.52* and *A1.53*, given 1 Rb.U. daily and killed on the 31st day, showed secretory exhaustion of the glands to a marked degree. This was particularly true of *A1.53* (fig. 1). The glands were straight and narrow, the nuclei in the thin epithelium were elongated and occupied most of the space in the cells, and no indications of secretion were present. The general condition resembled that of post-castration atrophy. Although these 2 animals received identical treatment, the endometrium of *A1.52* was better developed. The epithelium of the distal parts of the glands in the compactum was only a little thicker than the greatest diameter of the slightly elongated nuclei, yet a few glands showed some secretion. The basal portions of the glands were greatly dilated, and the epithelium was considerably thicker, and showed a moderate amount of secretion. It is interesting that such endometria should require 1 Rb.U. of progesterone daily to maintain them, and that they bleed when the dosage is reduced to ½ Rb.U. daily.

When the estradiol and progesterone were given simultaneously for the inhibition of bleeding, the smallest dosage used was 25 R.U. plus ¼ Rb.U. A biopsy was taken from the uterus of *A1.65* on the tenth day while receiving such treatment. The glands of the endometrium (fig. 4) were moderately dilated, coiled, and some secretion present in the extreme outer ends of a few glands. The nuclei of the epithelium, in the distal part of the glands, were vesicular and arranged in a single row separated from the basement membrane by a clear zone containing glycogen. Towards the base the nuclei became elongated and irregularly placed. The development of the endometrium was comparable to the presecretory condition found soon after the beginning of the luteal phase of a normal cycle.

It was not possible to determine whether or not a combination of 25 R.U. plus ¼ Rb.U. was capable of producing a fully formed premenstrual condition, as this dosage inhibited bleeding for only a maximum of 14 days. However, when a combination of 50 R.U. plus ¼ Rb.U. was given, bleeding was postponed in one animal for 28 days. A biopsy of the uterus of this monkey, *A1.68*, was taken on the 21st day. When the endometrium (fig. 5) was compared with that of *A1.65*, the glandular epithelium in general was thinner, and in places where the glands were slightly dilated the vesicular nuclei were arranged in a row, whereas at other places in the compactum the nuclei, although

[4] It is realized that the terms used for describing the secretory condition of the uterine glands are perhaps inaccurate. Probably at no time is secretory activity totally absent, and our designations are terms of degree. By presecretory we mean the condition found between the time of the first indication of a luteal effect and the initiation of fraying of the luminal ends of the epithelial cells, the latter being considered the beginning of the secretory phase. Secretory exhaustion is used to describe the condition of relative inactivity of the glands found after a prolonged treatment with progesterone.

spherical, were irregularly placed. Secretion was going on in only a few glands, and the general appearance was that of inactivity.

The 2 animals, *A1.67* and *A1.58*, receiving 25 R.U. plus ½ Rb.U. and 50 R.U. plus ½ Rb.U. respectively, with biopsies taken on the 21st and 22nd days, had premenstrual endometria somewhat better developed than those of monkeys given ¼ Rb.U. in the combination. This was particularly true of the lumina in the basal part of the glands, which were conspicuously larger (fig. 6, 7). There was very little secretory activity, some being present in the basal portions of part of the glands in *A1.67*, whereas in the endometrium of *A1.58* secretion was almost entirely absent.

The histology of the endometria show clearly that the minimal dosage of progesterone (¼ Rb.U.) used in these experiments is sufficient to produce unquestionable premenstrual changes when given simultaneously with estrin. The differences in response are those of degree, and seem to be correlated with length of treatment and dosage. It has been found previously in similar experiments (3) that about 10 days are required to develop an endometrium to a secretory condition, even when much larger amounts of progestin are employed. In this regard it is of interest to compare the endometrium of *A1.65* (fig. 4); receiving 25 R.U. plus ¼ Rb.U. daily for 10 days, with that of *A1.62* (fig. 8) given 100 R.U. plus 1 Rb.U. daily for the same period. Both endometria were in what is commonly termed a presecretory condition.

When the injections were continued for about 20 days, the endometria seemed to have at least one characteristic in common, namely a conspicuous decrease in secretory activity. This appears equally true for those given a combination of the two hormones and those on progesterone alone. A similar observation has been reported elsewhere (3, 6) for monkeys that were given larger doses of progestin alone and in combination with estrin. Secretion may begin in the extreme outer ends of the glands on about the tenth day of progesterone treatment and progress towards the base, and by about the 20th to the 25th days secretion, if present, is limited to the dilated, basal portions of the glands. The material in the present experiments was not selected at graded intervals sufficiently close to follow the secretory changes, but the end results on the 20th day are similar to those previously observed. These observations suggest the possibility that the secretory changes in the endometrium require about the same length of time for completion, whether the dosage of progesterone is large or just above the threshold required for stimulation.

Glycogen. The presence of glycogen in the endometrial epithelial cells is generally associated with the luteal phase of the menstrual cycle, but has not been reported as being present during the follicular phase. This makes it appear that progesterone is more concerned with glycogen formation and elimination than estrin. In earlier experiments (7), we found glycogen granules scattered along the basal ends of the epithelial cells when 80 to 100 R.U. of estrin were injected daily for 20 days or longer. Recent experiments have demonstrated that a dosage of 100 to 300 R.U. daily will invariably lead to glycogen storage. The amount of glycogen present in the glandular epithelium seemed to be correlated both with dosage and length of treatment. Monkey

A1.46 receiving 100 R.U. of estrin daily for 20 days had granules of glycogen distributed along the basement membrane of the epithelium of the glands, whereas the endometrium of *A1.56* after 38 days of similar treatment showed a much greater glycogen deposition. Such glands in ordinary histological preparations showed a clear zone between the nuclei and the basement membrane.

It has been mentioned previously (3) that one of the first indications of progesterone action, as seen in routine histological preparations, is the appearance of a conspicuous clear zone between the epithelial nuclei and basement membrane of the glands. It is now known that a similar zone can be produced by injecting large amounts of estrin, but not by comparatively small doses. The presence of this zone depends upon the deposition of glycogen in the epithelial cells, and consequently it is better developed when large doses of estrin are given.

From a histological standpoint there seems to be a conspicuous disparity between the amount of glycogen present in the epithelium and that found in the lumina of the glands. Even when the epithelial cells are loaded with glycogen, comparatively few granules are in the process of being released from the luminal ends of the cells. This suggests that estrin is more effective in stimulating the formation and storage of glycogen than in its release into the lumen, or that the glycogen escapes from the cells in some other form such as sugar, and is not detectable by the histological technics used.

In sharp contrast to the apparent storage of glycogen produced by estrin, progesterone brings about both glycogen formation and its rapid release from the cells as discrete particles. It is quite probable that the 'priming' dosage of 100 R.U. of estrin daily brought about an accumulation of some glycogen in the endometria of all the animals used in the present series of experiments, but it is interesting that following such treatment even small doses of progesterone induced a conspicuous unloading of glycogen into the lumina of the glands. A combination of 25 R.U. plus ¼ Rb.U. given daily for 10 days (*A1.65*) is as effective in this regard as 100 R.U. plus 1 Rb.U. (*A1.62*). The amount of glycogen being released into the uterine glands of these 2 monkeys is greater than that seen in the endometria of *A1.67*, *A1.68*, and *A1.58*, given 25 R.U. plus ½ Rb.U., 50 R.U. plus ¼ Rb.U., and 50 R.U. plus ½ Rb.U. respectively for 21 to 22 days. In fact, the monkeys on the longer treatment show comparatively little elimination of glycogen.

Another experiment not recorded in the table gives some indication that estrin, when administered in combination with small doses of progesterone, may enhance rather than suppress the action of progesterone. Monkey *A1.68*, after a 'priming' treatment of 100 R.U. daily for 20 days, was given 200 R.U. plus ½ Rb.U. daily for 19 days. The glycogen being formed and discharged into the uterine glands of this animal was greater in amount than that observed in the endometria of *A1.67* and *A1.58*, although they received the same daily dosage of progesterone for 21 and 22 days, but only 25 R.U. and 50 R.U. of estrin daily. The idea that estrin may aid in this process is also supported by the experiments in which only progesterone was used. Monkey *A1.60* receiving

¾ Rb.U. of progesterone daily for 19 days showed less glycogen in the lumina of the glands than *A1.68* given 200 R.U. plus ½ Rb.U. for the same length of time. The data, however, are too few to decide this point definitely.

The elimination of glycogen from the glandular epithelium is correlated with the secretory activity of the glands. We have mentioned that the uterine glands, when progesterone is given alone, or when a combination of progesterone and estrin are injected, pass through a period of active secretion and, if the treatment is prolonged, reach a condition that we have called secretory exhaustion. Likewise, glycogen elimination attains a peak during the secretory phase and decreases with the secretory exhaustion of the glands.

Both estrin and progesterone produce a deposition of glycogen in a few large cells scattered throughout the stroma. These cells are usually widely separated, have large vesicular nuclei, and their distribution shows no definite position with regard to other structures of the endometrium. They are more numerous in some endometria than others, but the variation does not seem to be correlated with the nature of the treatment given.

DISCUSSION

It is difficult to determine the individual rôles played by estrin and progesterone in the development of the endometrium and the inhibition of bleeding, when both hormones are given simultaneously. It is true that dosages of either hormone which will not postpone bleeding will do so when given together. Also, an amount of progesterone (about ¼ Rb.U.) that is insufficient to delay bleeding perhaps has little effect on the endometrium when given alone, and it is only when a dosage is employed sufficiently large to give definite inhibition (about ½ Rb.U.) that unquestionable histological changes in the glands can be detected. These results may be explained on the basis of time, that is, the M.E.D. of progesterone must be one that inhibits bleeding longer than the minimal time required for histological changes.

The experiments reported here show that ¼ Rb.U. of progesterone, when combined with 50 R.U. of estrin (*A1.68*, fig. 5) will produce definite although mild premenstrual changes in the endometrium by the 21st day. It is yet a question as to whether these histological changes are due to a synergism between the two hormones, or to the fact the small doses of progesterone were able to produce these effects when the inhibition provided a longer time for action. Yet other experiments have shown (6) that, when larger amounts are used, better premenstrual development of the endometrium occurs when the two hormones are combined than when the same dosage of progesterone is given alone.

There is no definite indication in these experiments that estrin inhibits the action of progesterone on the endometrium. In fact, one animal (*A1.68*) given 200 R.U. plus ½ Rb.U. daily for 19 days had endometrial development comparable to that of *A1.58* (fig. 7) which received 50 R.U. plus ½ Rb.U. daily for 22 days. The only obvious difference is the amount of secretion and glycogen elimination, both being greater in *A1.67*, which may be within the expected range of variation. However, it would be beyond the scope of our

data to imply that larger amounts of estrin may not inhibit an effective dose of progesterone.

The experiments discussed in this paper and elsewhere (3) indicate that the threshold dosage of progesterone necessary for the production of premenstrual changes in the endometrium is smaller than that required for the inhibition of bleeding.

That progesterone can inhibit bleeding after the discontinuance of an estrin treatment is a generally accepted fact, but the ability of estrin to inhibit bleeding when preceded by progesterone is not so well established. In the experiments reported by Engle et al (4), bleeding was not postponed by estrin following a series of injections of progestin. On the other hand Zuckerman (8) found that bleeding did not occur during a 40-day experiment in which 2000 I.U. of esterone was given daily for 14 days, followed by 500 I.U. of estrin plus 2 Rb.U. of progesterone daily for 12 days and continued on 500 I.U. of estrin daily. It has been observed that the inhibition of post-castration bleeding by estrin can be accomplished quite easily when the ovaries are removed during the follicular phase of the cycle, but becomes progressively more difficult with the development of a corpus luteum (3).

When Corner (9) attempted to maintain the normal estrin level during the cycle by injecting from 12 to 90 R.U. of ketohydroxyestrin or the monobenzoic ester of dihydroxyestrin thrice weekly, he found that such treatment did not postpone the onset of the next menstruation but, if continued, did delay subsequent menstrual periods. However, he discovered that the cycles of 3 monkeys out of a group of 6 were definitely prolonged by the injection of 1 Rb.U. of progesterone per day. Zuckerman (10) gave an average daily dose of approximately 500 R.U. of estrin (ketohydroxyestrin) during the last half of 7 cycles of a Mangabey and during two of a Macaque monkey, without disturbing the normal menstrual rhythm.

It seems quite probable that these experimental results for monkeys may be applicable to the human being. Fluhmann and Hoffmann (11) found that total dosages of 3000 to 9000 R.U. of estrin administered to 12 women over a period of eight to 12 days did not affect the duration of the cycle, the length of the period, or the amount of blood loss, whereas Kaufmann (12) has demonstrated, by 2 types of experiments, that estrin cannot prevent menstruation from a progestational endometrium following withdrawal of progesterone.

These observations support the idea that once an endometrium has been converted into a premenstrual condition by progesterone it is extremely difficult, if not impossible, to inhibit menstruation by giving estrin following the withdrawal of progesterone. However, it can be seen in table 1 that bleeding was inhibited by estrin after progesterone had been injected in sufficient amounts to cause definite modifications of the endometrium. We have mentioned that ½ Rb.U. of progesterone daily, when combined with 25 R.U. or 50 R.U. of estrin, will produce marked premenstrual changes in the endometrium (*A1.67*, fig. 6 and *A1.58*, fig. 7) and that the effects of ¼ Rb.U. plus 50 R.U. are moderate (*A1.68*, fig. 5). Bleeding after discontinuance of such treatments was not inhibited by 50 R.U. of estrin in 2 experiments (*A1.68* and

A1.56) or by 100 R.U. in another (*A1.58*), but in 3 animals out of 4 given 500 R.U. menstruation had not occurred by the end of 10 to 18 days (*A1.52, A1.55, A1.52* and *A1.62*). A uterine biopsy from one of these animals (*A1.55*, fig. 9) indicated that the premenstrual condition, which presumably existed at the beginning of the estrin treatment, had been changed over into one having characteristics typical for estrin action at high dosages. Also bleeding was further inhibited in *A1.52* and *A1.62* for 10 days by giving 100 R.U. plus 1 Rb.U. daily, and a uterine biopsy (fig. 8) showed that a luteal effect was reëstablished. These experiments demonstrate that it is possible to convert an estrin endometrium to a premenstrual, back to an estrin, and again to a premenstrual condition without the intervention of bleeding.

These experiments also suggest that the dosage required of either hormone alone or in combination, to prevent bleeding, may depend upon the nature of the treatment which immediately precedes. For example, 50 R.U. plus ½ Rb.U. postponed bleeding when preceded by a 'priming' dose of 100 R.U. of estrin daily for 20 days (*A1.55*), but bleeding occurred on the seventh day when the same combination was given following a 10-day treatment of 500 R.U. daily. Also, 500 R.U. of estrin daily inhibited bleeding when preceded by 25 R.U. plus ½ Rb.U. or 50 R.U. plus ½ Rb.U. daily, but failed to do so when given after a combination of 100 R.U. plus 1 Rb.U. The influence of the amount of estrin used as a 'priming' dose on the quantity of progesterone required for subsequent inhibition of bleeding, and the relation between the dosage of a preceding progesterone treatment and the amount of estrin required to postpone bleeding are somewhat outside the scope of these experiments, but they nevertheless suggest that such relationships do exist.

The secretory condition of the uterine glands cannot be used as a criterion for determining whether estrin will inhibit bleeding, following progesterone withdrawal. The experiments reported here show that 25 or 50 R.U. plus ½ Rb.U. daily will produce a typical premenstrual reaction in the glands within about 20 days (fig. 6, 7), and that 500 R.U. daily will prevent bleeding from such endometria. On the other hand, 100 R.U. plus 1 Rb.U. daily elicits only a presecretory condition in 10 days (fig. 8), yet 500 R.U. daily does not prevent bleeding after such treatment. Also, a group of 6 castrated monkeys, not shown in the accompanying table, which received 100 RU. of estrin daily for 20 days and 100 R.U. plus 1 Rb.U. daily for 5 days began bleeding between 60 and 80 hours after the withdrawal of progesterone, even though the estrin injections were continued. This experiment was repeated on 4 animals, with the exception that 100 R.U. plus 1 Rb.U. was given daily for 3 days and bleeding started between 85 and 110 hours. It seems quite improbable that the uterine glands of these animals could have shown more than slight progesterone effects. In fact, our material (see also 3) indicates that 1.0 to 4.0 Rb.U. of progesterone daily, when given alone or with estrin, rarely produces more than a presecretory condition by the tenth day, and a fully developed premenstrual endometrium usually does not appear before about the 15th day. It is clear that the effects of progesterone which make it difficult to inhibit bleeding by subsequent injections of estrin occur independent of, and

much earlier than, the glandular changes. The failure of estrin to prevent bleeding may be due to a modification of the stroma by progestin. The suggestion by Bartelmez (13) that probably the true explanation of menstruation may be found in the circulatory changes in the endometrium seems a good one, and is supported by the recent work of Daron (14).

SUMMARY

Castrated monkeys given 100 R.U. of estrin daily for 20 days will bleed soon after discontinuance of the treatment. Such uterine bleeding is not postponed beyond the expected time by 25 R.U. of estrin daily, or ¼ Rb.U. of progesterone daily. Bleeding is inhibited for 9 to 10 days by 50 R.U. of estrin daily. One-half Rb.U. of progesterone daily will postpone bleeding for 8 to 13 days; ¾ Rb.U. for 15 to 25 days; 1 Rb.U. for as long as 44 days, after which bleeding will occur within 4 to 7 days if the dosage is reduced to ½ Rb.U. daily. When estrin and progesterone are injected simultaneously, 25 R.U. plus ¼ Rb.U. daily will inhibit bleeding for 8 to 14 days; 25 R.U. plus ½ Rb.U. for at least 24 days; 50 R.U. plus ¼ Rb.U. for at least 28 days; and 50 R.U. plus ½ Rb.U. were given for as long as 22 days without indications of bleeding.

Amounts of progesterone which do not inhibit bleeding long enough to permit the development of a premenstrual endometrium, when given alone following an estrin treatment, will produce a premenstrual reaction when given in conjunction with a suitable dosage of estrin. Fifty R.U. of estrin plus ¼ Rb.U. of progesterone daily will elicit a definite progestational reaction within 22 days, while 25 or 50 R.U. plus ½ Rb.U. daily for the same length of time will produce a fully developed premenstrual condition. Bleeding from such endometria is not postponed by 50 or 100 R.U. of estrin daily, but may be inhibited by 500 R.U. daily. When bleeding from an endometrium which has undergone premenstrual development is inhibited by estrin (500 R.U. daily), the condition is changed back into that typical of estrin action. Such endometria may be again transformed into a premenstrual state by giving 100 R.U. plus 1 Rb.U. daily, after which bleeding cannot be inhibited by 500 R.U. of estrin daily. Thus an endometrium showing the effects of estrin can be changed to a premenstrual, back to an estrin, and again to a premenstrual condition without the intervention of bleeding.

Considerable glycogen is found in the uterine glands when 100 R.U. of estrin is given daily for 20 days or longer, and when larger doses are injected for the same period there is a correspondingly greater deposition of glycogen. Although the epithelial cells may contain an abundance of glycogen as a result of the action of estrin, very little is released into the lumina of the glands at least in a stainable form. In contrast with this, progesterone produces both the formation and release of glycogen. The discharge of glycogen from the glandular epithelium reaches its height during the secretory phase, and decreases as the endometrium approaches the condition of secretory exhaustion.

Both estrin and progesterone produce a deposition of glycogen in a few large cells scattered throughout the stroma. Such cells are very few when 100 R.U. of estrin is injected daily for 20 days, but are always present following

treatment with larger doses of estrin, progesterone, or a combination of the two hormones.

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NUTRITIONAL REQUIREMENTS OF PREGNANT AND LACTATING RATS STUDIED BY THE SELF-SELECTION METHOD

CURT P. RICHTER AND BRUNO BARELARE, JR.
From the Psychobiological Laboratory, Henry Phipps Psychiatric Clinic, Johns Hopkins Hospital
BALTIMORE, MARYLAND

In a previous study the self-selection technic was used to determine the nutritional requirements of normal animals (1,2). Pure substances rather than so-called natural foods or food mixtures were offered for choice. It was found that, given a selection of 11 substances in separate containers (casein, sucrose, olive oil, sodium chloride, dibasic sodium phosphate, calcium lactate, potassium chloride, dried baker's yeast, cod liver oil, wheat germ oil, and water), rats made selections which were conducive to excellent growth and normal reproduction.

This self-selection technic has now been used to study the nutritional requirements during pregnancy and lactation.

METHODS

The rats were kept separately in cages, a cross-section of which is shown in figure 1. One part (*C*) of the cage ($9\frac{1}{4}'' \times 9\frac{1}{4}'' \times 5\frac{1}{2}''$) contained the 3 food cups for the solids, and 8 graduated inverted bottles for the different mineral solutions, oils and water. The other part (*E*), $12'' \times 8'' \times 11''$, contained the rat's nest and served as a living compartment. These two parts were hung from hooks on each side of a central metal partition (*B*); they were connected by a hole $2\frac{1}{2}$ inches in diameter.

In order to keep the litter animals away from the mother's food cups and mineral bottles, and at the same time within easy reach for the mother, it was necessary to modify the living cage (*E*). A partition (*D*) was placed in it, separating the nest and litter compartment (*A*) from the food and minerals compartment (*C*). A hole, $1\frac{1}{2}$ inches wide, at the top center of the partition made it possible for the mother to pass freely back and forth between the litter and her food. She learned to jump through this hole at once, but the young animals were unable to reach it until they were at least 25 days old, when they were ready to be weaned.

The 3 food cups contained casein (purified and autoclaved), sucrose, and dried baker's yeast; the 8 bottles contained solutions of sodium chloride, 3 per cent, dibasic sodium phosphate, 8 per cent, calcium lactate, 2.4 per cent, potassium chloride, 1 per cent, olive oil, cod liver oil, wheat germ oil, and water. Readings of the food and fluid intake and of the vaginal smears were made daily; body weight was recorded weekly. The bottles were cleaned and refilled at least twice weekly.

The 10 animals used in the experiments were kept on this multiple choice diet for at least a month before they were mated. At the end of this time they all showed normal growth and regular 4- to 5-day estrous cycles. They all gave birth to normal young, and nursed them during the usual lactation period.

RESULTS

A). Fat, protein, and carbohydrate appetite. The results of these experiments are summarized in the curves in figure 2, which give the average daily in-

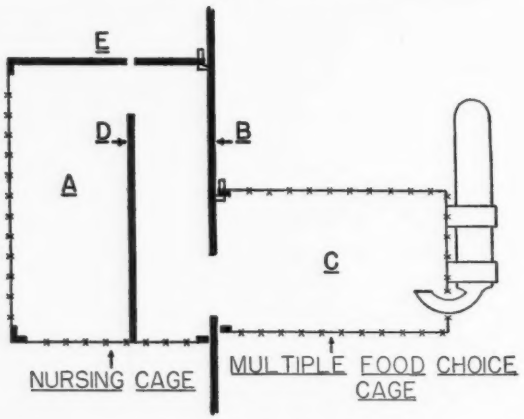

Fig. 1. Cross-section of cage, showing the separate compartments used for nursing and feeding.

take of olive oil, casein, and sucrose for the 10 females in 5-day periods for 20 days before mating, 20 days of pregnancy, 25 days of lactation, and 10 days after weaning.

Fat (Olive oil). In figure 2*A* it will be seen that the average daily intake increased from 1.2 cc. before mating to 2.1 cc. at the end of pregnancy, and

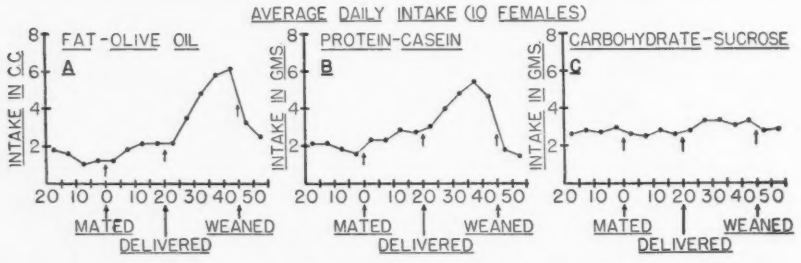

Fig. 2. Curves showing average daily intake for fat, carbohydrate, and protein in 5-day periods for 20 days before mating, for 20 days of pregnancy, for 25 days of lactation, and for 10 days after weaning.

to 6.1 cc. at the end of lactation. After weaning it dropped to 2.4 cc. The highest individual average daily intake for the last 5-day period of pregnancy was 5.2 cc. and for the last 5-day period of lactation 13.0 cc.

Protein (Casein). An almost equally great increase was present in the

casein appetite (fig. 2*B*). The average daily intake increased from 1.6 gm. before mating to 2.7 gm. for the last 5-day period of gestation, and to 4.6 gm. for the last 5-day period of lactation. After weaning the intake returned to the pregestational level. The highest individual average daily intake was 5.2 gm. for the last 5-day period during pregnancy and 7.8 gm. for the last 5-day period during lactation.

Carbohydrate (*Sucrose*). In marked contrast, the sucrose appetite showed

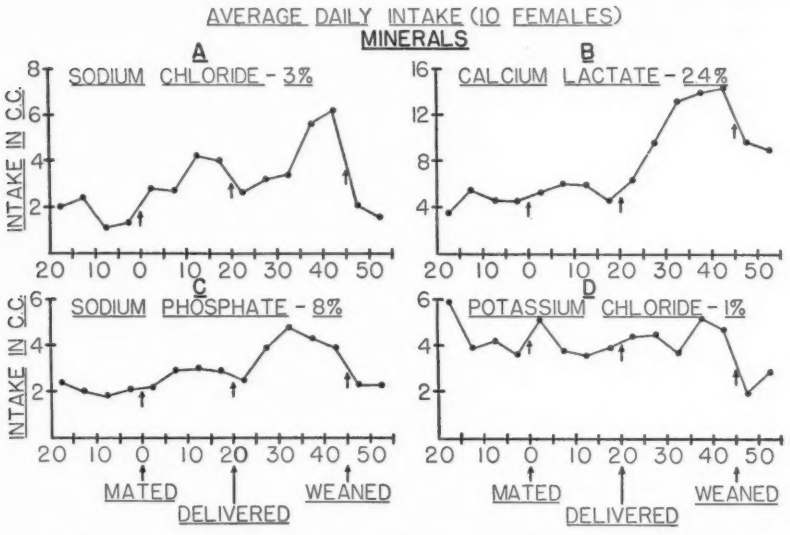

Fig. 3. Curves showing average daily intake of the various mineral solutions in 5-day periods for 20 days before mating, for 20 days of pregnancy, for 25 days of lactation, and for 10 days after weaning.

practically no change during pregnancy, and only a slight increase during lactation (fig. 2*C*).

B). Minerals. Sodium chloride, 3 per cent. (fig. 3*A*). A definite increase in the sodium chloride intake was present as early as the first 5 days of gestation. At the end of gestation it had increased from a level of 1.4 cc. to 4.0 cc.; at the end of lactation it had reached a level of 6.0 cc. Weaning produced an immediate decrease to the normal level. This is in agreement with results reported previously (3). The highest individual average daily intake was 9.0 cc. for the last 5-day period during pregnancy, and 14.2 cc. for the last 5-day period during lactation.

Calcium lactate, 2.4 per cent (fig. 3*B*). The calcium lactate appetite increased from an average daily intake of 4.4 cc. before mating to 6.0 cc. for the second and third 5-day periods of gestation, and then dropped back to 4.4 cc. during the last 5-day period of gestation. It increased very sharply during lactation, reaching a peak of 14.4 cc. in the last 5-day period. After weaning the decrease was gradual, as compared to the abrupt postlactation drop in sodium chloride intake, and did not reach its normal level for several weeks. The highest individual average daily intake during pregnancy was 10.0 cc.; during lactation 32.0 cc.

Dibasic sodium phosphate, 8 per cent. Figure 3*C* shows that the sodium phosphate appetite increased definitely during pregnancy, and even more markedly during lactation. After weaning it dropped almost at once to its pregestational level. The highest individual average daily intake during pregnancy was 6.9 cc., and during lactation 10.8 cc.

Potassium chloride, 1 per cent. In comparison to the 3 previous minerals, the appetite for potassium chloride showed irregular fluctuations. Figure 3*D* shows that the intake remained on a fairly constant level during pregnancy and lactation. Of particular interest, however, is the fact that after weaning it dropped quite abruptly below the pregestational level, falling in one animal from 18.2 cc. to 3.6 cc.

C). Vitamins. Vitamin B complex (dried baker's yeast). (fig. 4*A*). Although it was slight in comparison with the increase in intake of olive oil, casein, and minerals, the increase in the yeast appetite was clearly noticeable

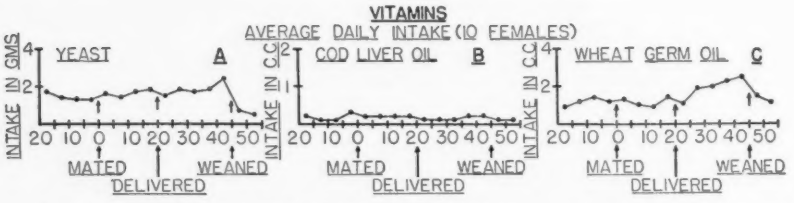

Fig. 4. Curves showing average daily intake of the vitamin substances (yeast, cod liver oil, wheat germ oil) in 5-day periods for 20 days before mating, for 20 days of pregnancy, for 25 days of lactation, and for 10 days after weaning.

during pregnancy, and even more marked during lactation. After weaning the average daily yeast intake showed a very sharp drop from 2.4 to 0.4 gm. In one animal the intake dropped from 7.4 to 0 gm. The highest individual average intake during pregnancy was 3.2 gm. per day and during lactation 7.4 gm. per day.

Vitamins A and D (cod liver oil). The changes in the cod liver oil appetite were somewhat difficult to determine, due to the small quantities ingested. It is clear that no changes took place at any time except possibly during the first 15 days of lactation and the first 10 days of weaning (fig. 4*B*). The daily appetite was extremely small, when compared to that of vitamin A and D deficient animals, which sometimes drank as much as 5 cc. of cod liver oil per day until the deficiency was corrected. The highest individual average daily intake during pregnancy was 1.2 cc.; during lactation 0.7 cc.

Vitamin E (wheat germ oil). The appetite for wheat germ oil remained practically unchanged during pregnancy, but showed a definite increase during lactation (fig. 4*C*). The highest individual average daily intake during lactation was 6.6 cc.

Individual differences in changes in appetite. In the 10 animals the individual differences in the changes in appetite were practically negligible. At least 9 of the 10 animals showed an increased intake in casein, olive oil, sodium chloride, sodium phosphate, calcium lactate, and yeast, and little or no

change in the intake of sucrose. The appetite for potassium chloride was the only one which did not give consistent results. Five animals showed a decrease in potassium chloride appetite, and 5 an increase. After weaning, however, the results were much more uniform, in that all the animals showed a drop in intake to a point below the pregestational level.

Changes in caloric percentages of fat, carbohydrate, and protein. The results of these experiments are summarized in table 1, which gives the caloric percentages based on the average daily caloric intake during the last 5 days before mating, the last 5 days of pregnancy, the last 5 days of lactation, and the second 5-day period after weaning. Fats include olive oil, cod liver oil, and wheat germ oil; carbohydrates include sucrose and the yeast carbohydrate; and proteins include casein and the yeast protein.

Before mating, fat contributed 52.6 per cent of the total calories, at the end of pregnancy 54.3 per cent, and at the end of lactation 65.0 per cent.

TABLE 1. PREGNANCY AND LACTATION. AVERAGE DAILY INTAKE (10 FEMALES)

	Normal	Pregnancy	Lactation	After weaning
	Average Caloric Percentages			
Fats	52.6	54.3	65.0	61.6
Carbohydrates	25.1	17.1	11.3	21.1
Proteins	22.3	28.1	23.8	17.3
	Average Total Daily Intake, in Calories			
Self-selection	45.3	59.8	118.3	52.3
McCollum	56.0	60.0	160.0	56.0
	Average Daily Weight of Intake, in Grams			
Self-selection	8.8	11.3	19.8	9.0
McCollum	14.0	15.0	35–45	14.0
	Average Daily Water Intake in Cc.			
Self-selection	20.7	30.0	36.4	19.6

Carbohydrate caloric intake decreased from 25.1 per cent before mating to 17.1 per cent at the end of pregnancy, and to 11.3 per cent at the end of lactation. Protein caloric intake increased from 22.3 per cent before mating to 28.1 per cent at the end of pregnancy, and then dropped to 23.8 per cent at the end of lactation. Actually, as was shown above, protein increased markedly during lactation, but its caloric percentage increase was outweighed by the greater increase in the fat intake.

Total caloric intake. The changes in average daily total caloric intake during pregnancy and lactation are shown in table 1. The caloric intake increased from a daily average of 45.3 calories before mating to 59.8 at the end of pregnancy, to 118.3 at the end of lactation, and then dropped to 52.3 after weaning.

Compared with the caloric intake of animals on the standard McCollum diet (4), the caloric intake on the self-selection diet was markedly lower throughout. On the McCollum diet the caloric intake increased from 56.0 cal. before mating to 60.0 during pregnancy, to 160.0 cal. during lactation (first 15 days), and then dropped to 56.0 after weaning.

Changes in total weight of solids. The average daily weight of the solids (including oils) increased from 8.8 gm. before mating to 11.3 at the end of

pregnancy, to 19.8 gm. at the end of lactation, and dropped to 9.0 gm. after weaning (table 1).

A comparison with the weight of the daily solid intake on the McCollum diet shows that the weight differences were even greater than the differences in caloric intake. The weight of the food on the McCollum diet increased from 14.0 to 15.0 gm. at the end of pregnancy, to 35-45 gm. during lactation (4), and then dropped to 14.0 after weaning.

Of particular interest is the fact that on the self-selection diet the increase in calories was greater than the increase in weight (fig. 5). During lactation the percentage increase in calories was 161, whereas the percentage increase in weight was only 125. This difference is due to the increased intake of fat.

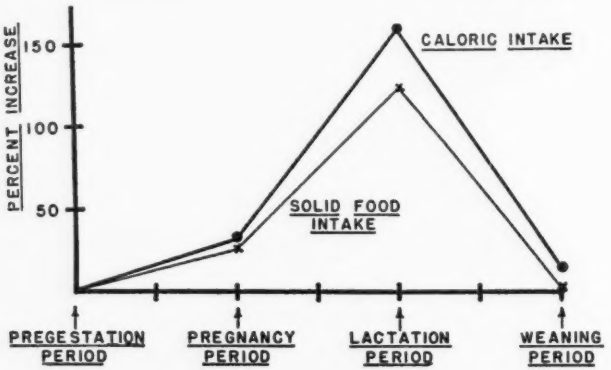

Fig. 5. Curves showing the percentage increase in terms of caloric intake during the 4 periods, as compared to the percentage increase in grams of solid food ingested.

Change in water intake. The total fluid intake increased from 20.7 cc. per day before mating to 30.0 cc. at the end of pregnancy, and to 36.4 cc. at the end of lactation, and then dropped to 19.6 cc. in the postlactation period (table 1). The increase during lactation was fairly large, but still it seemed surprisingly small considering the amount of water which must leave the body in the milk. The low water intake may be explained, at least in part, by the high fat content of the diet, since we have found that water intake of animals on a high fat diet is always markedly reduced.

SIGNIFICANCE OF CHANGES IN APPETITE

A). Protein, Fat, and Carbohydrate. The increased demands for protein and fat during pregnancy and lactation are in keeping with our knowledge of the needs of the growing fetuses and young. We know that protein is needed for the building of new tissue and that fat is needed for the storage of energy.

The lack of any change in carbohydrate appetite is contrary to the current beliefs of obstetricians regarding the increased carbohydrate needs of pregnant and lactating women. The absence of any increase is not an unexpected

result when one takes into account the reduction in activity of the pregnant and lactating animals, and hence the reduced needs for immediate energy. Wang (4) showed that pregnant and lactating rats are about 90 per cent less active than normals.

It will be important to repeat these experiments with lactose and galactose. It is possible that lactating rats would show increased appetites for these two carbohydrates where they fail to show any changes in appetite for sucrose.

B). Minerals. The greatly increased demand for sodium chloride indicates needs which have not received much attention in metabolism studies of pregnancy and lactation. We do not know what part this increased salt intake plays. The fact that the increase appears within the first few days after mating, when the embryos are still minute, indicates that it must have a maternal rather than a fetal origin. The fact that a similar increase in salt appetite appears after adrenalectomy suggests that the adrenal glands may play an important part even in the first few days of pregnancy. It has been reported (5) that in pregnant guinea pigs the adrenal cortex first shows signs of stimulation, then signs of degeneration which become even more marked during lactation. It is also possible that the increased salt appetite may be directly dependent on changes in the ovaries, since it has recently been shown by Thorn and Harrop (6) that a close relationship exists between salt metabolism and ovarian function.

The increased calcium appetite is in agreement with our knowledge of the calcium needs for the building of bone and teeth of the growing fetuses and young. These experiments show that the calcium needs are much greater during lactation than during pregnancy; in fact, they are so great that the mothers were unable to supply the necessary calcium from their intake alone, making it necessary for them to call on their own calcium stores. That the mother's own supply was actually depleted is shown by the fact that the calcium intake did not return to its normal level until several weeks after weaning. The amount of calcium that could be obtained from a 2.4 per cent solution of calcium lactate without ingesting too large quantities of water apparently was not sufficient to spare the mother's own supply.

A similar increase in calcium appetite is known to occur after parathyroidectomy. This suggests a connection between the parathyroid glands and pregnancy and lactation. Actually it is known that the parathyroids do play an important part in these conditions, for very serious results (tetany, abortion, etc.) follow when these glands are removed either during pregnancy or lactation (7).

The increased phosphorus demands are in keeping with what is known regarding the needs of phosphorus in the building of bones, teeth, and blood in the growing fetuses and young. During pregnancy the increase in phosphorus intake was greater than that in calcium intake, whereas during lactation the increase in calcium was far greater than that in phosphorus. The importance of phosphorus in the metabolism of pregnancy and lactation is well known from the occurrence of symptoms of osteomalacia in cattle and in humans during pregnancy and lactation.

The absence of any changes in the potassium appetite during pregnancy

and lactation was surprising, in view of the constantly increasing potassium content of the intracellular fluids of the growing fetuses and young. The fact that the potassium appetite dropped so sharply after weaning, however, indicates that it may play some rôle. It is possible that this decrease may be due to the amount of potassium obtained from the uterus during the process of involution. In some way it may also be related to the increased calcium appetite still present after weaning. Evidence for this interrelationship between the potassium and calcium appetite may be found in the fact that the intakes of potassium chloride and calcium lactate return to normal about the same time, that is, approximately 3 weeks after weaning.

C). Vitamins. The changes in yeast demands are difficult to interpret, due to the afore-mentioned complex nature of yeast. The slightly increased intake during pregnancy and lactation may indicate increased needs for vitamin B_1, riboflavin, nicotinic acid, or even for protein. The fact that the yeast intake decreased so sharply after weaning to practically zero level certainly indicates that it played some special part during pregnancy and lactation. From preliminary observations it seems likely that the yeast appetite is largely dependent on the vitamin B_1 and riboflavin content (8). This can only be determined, however, by further experiments in which vitamin B_1, riboflavin and nicotinic acid are offered separately.

The demand for vitamins A and D as reflected in the cod liver oil appetite apparently are not greatly altered during pregnancy and lactation. The fact that vitamin A can be so readily stored makes it possible for adequately fed animals to maintain themselves by taking only minute quantities daily. It has been pointed out that vitamin A and vitamin D deficient rats drink as much as 3 to 5 cc. of cod liver oil per day, for several days in succession. There was no indication of any such demand in any of our normal animals.

It is not possible to draw any conclusions from our present experiments regarding the vitamin E demands during pregnancy and lactation. It is seen that the wheat germ oil intake is not increased during pregnancy, but shows an increase during lactation. The latter increase may simply be a manifestation of the increased demand for fat.

DISCUSSION

That the rats discriminated between these various substances brings further proof for the existence of special appetites for fat, protein, and carbohydrate, as well as for the minerals, sodium, calcium, phosphorus, and potassium. One of the most striking results of these experiments was the large increase in fat intake during pregnancy and lactation. In the previous paper (1) it was shown that normal non-pregnant rats show a great appetite for fat; now it is known that their intake during lactation may become so great that it provides as much as 65 per cent of the calories in the diet.

The high efficiency of the self-selection diet, as compared to the McCollum diet, merits special comment. Clearly, the smaller bulk and lower caloric intake show that the choice of purified substances makes it possible for the animal to satisfy its needs without ingesting large amounts of useless material.

The shortcomings of the present experiments hardly need special comment. Despite the consistency of the choices, the number of animals used was too small to warrant more than a preliminary statement regarding the nutritional requirements of rats during pregnancy and lactation. These experiments will have to be repeated, not only with a larger number of animals, but with a wider choice of minerals, including iron, magnesium, manganese, cobalt, copper and zinc. That these substances are necessary in the diet of normal animals is suggested by the fact that animals consistently ingest small amounts when they are offered in separate containers. For the same reason we feel that vitamin C should be offered. Also, the 3 chief components of yeast, vitamin B_1, riboflavin, and nicotinic acid will have to be offered separately.

The physiology underlying these changes in appetite is not understood. The amazing consistency of the choices made by the animals indicates that the changes do not depend on a trial-and-error process. It would seem far more likely that they are due to an altered chemistry of the taste mechanism, which reflects the altered chemistry of the rest of the body during pregnancy and lactation. Experiments are now in progress in which a study is being made of the appetite in animals whose taste nerves have been sectioned.

Finally, it would be of interest to know whether, on any kind of a self-selection basis, similar appetite changes would be shown by pregnant and lactating women; it would also be of interest to know what would be the result of increasing the fat intake at the expense of the present high carbohydrate intake. This would provide more calories per weight of food and thus diminish the amount of food ingested. It is possible that, by such an appetite study, new light may be thrown on the origin of various difficulties experienced by women during pregnancy and lactation.

SUMMARY

Ten female rats had shown, for at least a month previous to mating, normal growth and regular 4- to 5-day estrous cycles on a diet which they selected from 11 substances in separate containers. These animals gave birth to normal litters, averaging 8.5 young, and nursed most of them until they were separated 25 days later. The changes in appetite for the 11 substances were strikingly similar in the 10 animals. During pregnancy the appetite for casein (protein) and for olive oil (fat) showed a definite increase, whereas the appetite for sucrose (carbohydrate) showed a small decrease; the appetite for sodium chloride, sodium phosphate, and calcium lactate showed a marked increase, whereas the appetite for potassium chloride remained unchanged; the appetite for yeast increased slightly; there was no change in the appetite for wheat germ oil and cod liver oil.

During lactation the appetite for casein and olive oil increased to a higher level, whereas the appetite for sucrose still remained essentially unchanged; the appetite for sodium chloride, calcium lactate, and sodium phosphate also reached very much higher levels, whereas the appetite for potassium chloride showed no great alteration; the appetite for yeast and wheat germ oil increased slightly, but the appetite for cod liver oil remained unchanged. After weaning the appetite for casein, olive oil, sodium chloride, and sodium phosphate

dropped almost immediately to the pregestation levels; the calcium lactate appetite remained at a higher level for several weeks; the appetite for potassium chloride and yeast decreased sharply to subnormal levels.

The total number of calories increased from 45.3 per day before mating to 59.8 at the end of gestation and to 118.3 at the end of lactation. The caloric percentage of fats increased from 52.6 per cent before mating to 54.3 per cent at the end of gestation, and to 65.0 per cent at the end of lactation. Carbohydrate decreased from 25.1 per cent before mating to 17.1 per cent at the end of gestation, and to 11.3 per cent at the end of lactation. Protein increased from 22.3 per cent before mating to 28.1 per cent at the end of gestation, and to 23.8 per cent at the end of lactation. The total weight of the solid food intake (including oils) increased from 8.8 gm. per day before mating, to 11.3 at the end of pregnancy, and to 19.8 gm. at the end of lactation. The percentage increase in calories during pregnancy and particularly during lactation was considerably greater than the increase in weight of solid food.

If appetite may be used as a guide for nutritional needs, these results indicate a definitely increased need for protein and fat during pregnancy, and an even greater need during lactation. No increase in carbohydrate demand was noted. They also indicate increased needs for sodium chloride, sodium phosphate, and particularly for calcium lactate. Further evidence was thus obtained, demonstrating the ability of animals to make dietary selections which help to maintain a normal physiological equilibrium even during such altered states as pregnancy and lactation.

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A SIX-HOUR ASSAY FOR THE QUANTITATIVE DETERMINATION OF ESTROGEN[1]

E. B. ASTWOOD[2]
From the Biological Laboratories, Harvard University
CAMBRIDGE, MASSACHUSETTS

A study has been made of the effects of estrogen in the immature rat to attempt an inquiry into its mechanism of action (1). Preliminary experiments were concerned with the time relationships between an injection of estrogen, its earliest effects and the duration of the response. The early changes which occurred after a single injection were so striking that a detailed investigation of this effect was carried out. It was found that during the first few hours following an injection of estrogen the uterus of the immature rat undergoes a rapid change, accompanied by an increase in weight, due almost entirely to an accumulation of water.

The quantitative aspects of this response have been calibrated with sufficient precision to permit of its use as a simple method for the accurate assay of estrogenic substances, and it is the purpose of this paper to describe the assay method which has evolved. It will be necessary, however, to include a brief description of the reaction upon which the test is based.

METHODS

Female albino rats 21 to 23 days old were used; their weights ranged from 25 to 49 gm. with an average of 36 gm. All dosages of estrogen were dissolved in 0.1 cc. sesame oil and given in a single injection under the skin of the back. The animals were killed with chloroform and the uteri dissected out by cutting at the utero-tubal junctions, stripping off the mesometria and trimming the vagina from its attachment with the cervix. After blotting on absorbent paper the uteri were quickly weighed on a damped analytic balance. Water determinations were carried out by desiccating the weighed uteri in an oven at 110°C. The variation in the size of the animals used was partially corrected for by expressing all uterine weights in terms of the average body weight of the animals (36 gm.). This is not an entirely accurate correction as it tends to over-correct for animals at the extremes of the normal range.

Rate of Response. To determine the time relationships between the administration of estrogen and its subsequent uterine effect, large groups of rats were injected with 0.1 estradiol[3] and 5 to 10 animals killed at brief intervals of time thereafter; uterine weights, water and dry weights were obtained and histologic preparations made. Figure 1 shows the changes in weight and water distribution resulting from a single injection. During the first hour no effect was observed, but by the second hour a slight but definite increase in uterine

[1] Aided by a grant from the Rockefeller Foundation administered by Professor F. L. Hisaw.
[2] Rockefeller Fellow in the Natural Sciences.
[3] The crystalline estrone and estradiol were kindly supplied by Dr. Schwenk of the Schering Corporation.

weight had taken place. This increase in uterine weight proceeded rapidly during the next 3 hours and reached a maximum at 6 hours. Following this early maximum the weights declined somewhat, and by the 12th hour about one-quarter of the weight gained had been lost. A second increase in weight occurred during the next 15 hours, following which the uteri slowly returned to their normal resting state.

During the time of the early response the uteri appeared swollen, edematous and translucent; vascularity was somewhat increased and was especially prominent in the mesometria; the uteri themselves were quite pale. Water determinations confirmed the gross impression that the uteri were hydroptic, and showed that the early increase in weight was due almost entirely to an increased water

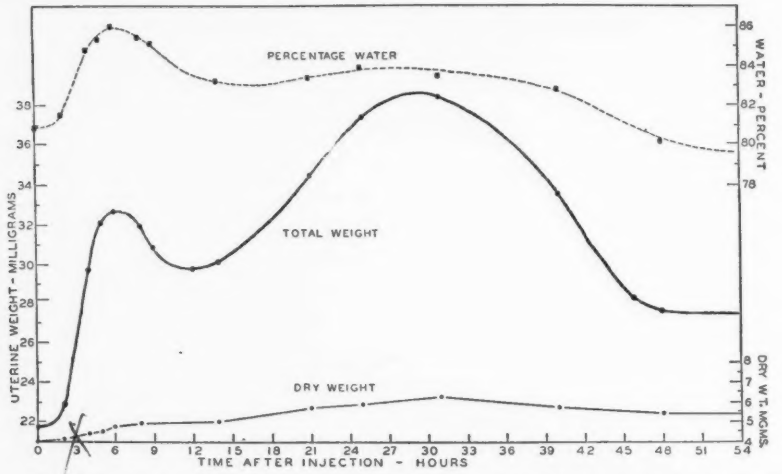

Fig. 1. Changes in percentage water, total weight and dry weight of immature rat uteri during 48 hours following a single subcutaneous injection of 0.1 γ estradiol in oil. The curves represent the average figures obtained from 370 animals.

content and that the fall that occurred at 12 hours was due to loss of water. The second weight increase represents a different type of effect, and, unaccompanied by a change in percentage water, indicates a true increase in protoplasm.

Figure 2 shows the rate of uterine change during the first 8 hours, and represents the average figures obtained for total weight, water and dry weight, from 30 groups of 5 to 10 animals each. It can be seen that very little increase in dry weight occurs, and that the 'growth' of the uterus is due very largely to an increased water content. That this shift in water is not attributable to an increased blood content is indicated by the appearance of the uteri, and proven by water determinations on the animal's blood. The average water content of normal untreated uteri, 80.8 per cent, increases to 85.8 per cent during the 6-hour interval (table 1). The water content of blood, 81.5 per cent, is entirely inadequate to account for this increase. These facts also indicate that there is very little increase in the quantity of blood in the uterus at this time, otherwise the dry weights of the uteri would be more appreciably raised.

Similar observations were made with larger dosages of estradiol. In each case a maximum was reached at 6 hours, and as much as 2.5 γ did not significantly alter the shape of the resulting 8-hour curve. Such high doses do influence the second rise, thereby effecting a greater late response.

Histologic study of the uteri showed that the maximal imbibition of water occurred in the endometrial stroma. At 6 hours this tissue appeared swollen by the accumulation of faintly staining material. The cells appeared to be larger, but whether the additional water confined itself to the cells or whether it also contributed to the intercellular materials could not definitely be determined. The subserosa was also somewhat affected, and, to a still lesser extent, the muscle layers. The epithelium showed no change, and no increase in mitotic

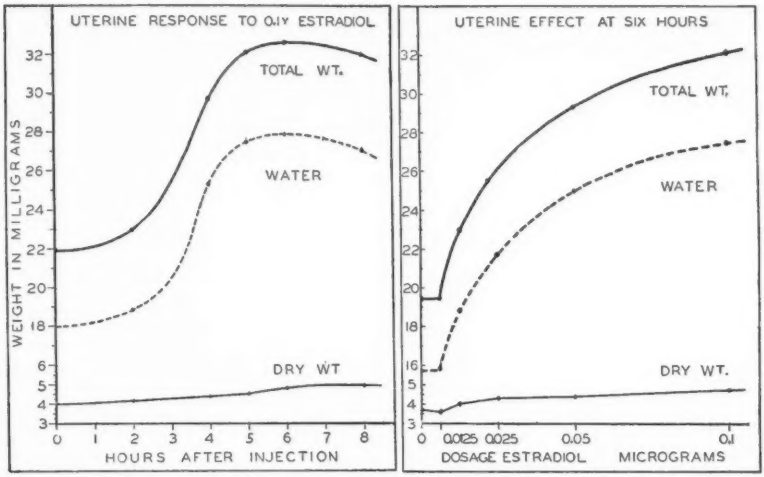

Fig. 2. Curves showing the increase in uterine weight, water and dry weight of immature rat uteri during the first 8 hours following a single subcutaneous injection of 0.1 γ estradiol.

Fig. 3. Showing the relation of total weight, water and dry weight of immature rat uteri 6 hours after a single injection of various dosages of estradiol. In this, as in figure 1, the dry weight scale is not continuous with the scale for total weight and water.

activity was observable during the first 6 hours. The increase in vascularity appeared to parallel the development of edema, and it was difficult to see which appeared first.

Effect of Dosage. From the curves of time relationships it was apparent that, over a wide dosage range, a maximal response occurred after 6 hours. Consequently a 6-hour interval was taken as a standard for subsequent studies of the effects of smaller doses. Groups of 5 to 10 animals were treated as before, and were killed 6 hours after a single injection of progressively diminishing doses of estradiol. Practically a maximal response was produced by 0.1 γ whereas below this dosage progressively lower uterine weights were obtained. Again it was found that the degree of weight increase was closely correlated with the amount of water accumulated in the uterus.

Figure 3 shows that a direct proportionality exists between average uterine weight and the dosage of estradiol, and that this relationship is largely con-

cerned with the extent to which various dosages affect the water content of the uteri. This quantitative agreement between dosage and response was obtained with such regularity that it has been used as a method for the rapid assay of estrogenic substances.

Assay Method. The earlier assays were done by determining the minimal amount of estrogenic substance which would give a maximal uterine effect in 6 hours. Although this method worked quite well, it had the disadvantage of striving for an end point, and gave no information regarding the excess hormone administered. Consequently a calibration curve was constructed from a large number of animals treated under standard conditions. The results obtained over a 6 months' period were collected, averaged and the extent of

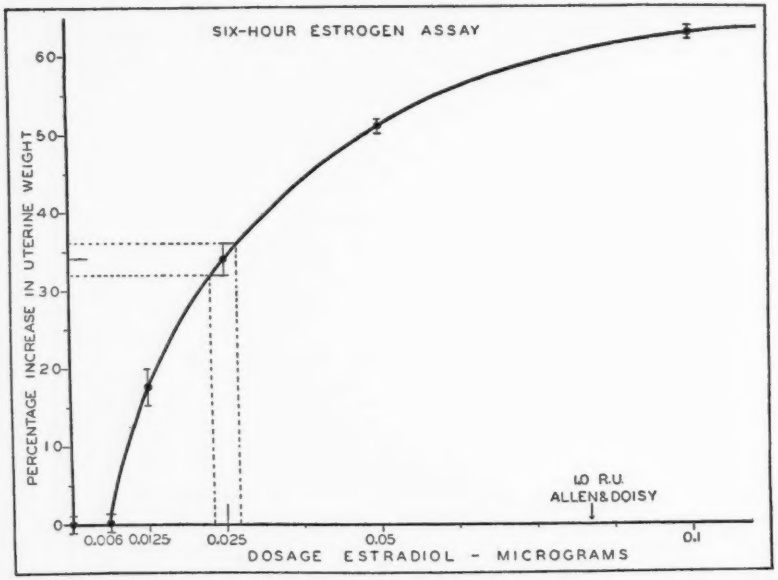

Fig. 4. Standardization curve for estrogen assay based on 300 immature rats, showing the relationship between dosage and percentage uterine weight increase. The standard deviations of the means are given as vertical lines above and below the points. The unit as defined in the text is shown to be equivalent to 0.025 γ estradiol. The Allen-Doisy unit is indicated for comparison.

variation determined. The standard curve thus obtained (fig. 4) established a direct logarithmic relationship between dosages of 0.006 γ and 0.1 γ estradiol and uterine weight. Dosages of 0.006 γ or below were without demonstrable effect, whereas above 0.1 γ the maximal dose was exceeded, and the relationship no longer held.

The percentage weight increase, resulting from a given dosage of estradiol, is thus predictable within a narrow range of variation, and conversely a given uterine effect may be used to indicate the quantity of estradiol administered. For purposes of comparison in this test, it is convenient to have some unit by which to designate estrogenic activity. Since there are a great number of different units already in use, an additional one would only lead to further confusion.

Consequently it seems advantageous to express the values obtained directly in equivalent weights of estradiol or estrone whereby comparisons can be made to the standard international unit.

The unit which has been arbitrarily adopted in this test is 0.025 γ estradiol. It is that amount of estrogen which, dissolved in 0.1 cc. sesame oil and injected subcutaneously into a group of 21 to 23-day-old rats, will produce a 32 to 36 per cent (average 34 per cent) increase in uterine weight in 6 hours. From figure 4 it can be seen that at this point the curve is quite steep and consequently the deviation of the mean, as graphically shown, is greater than at other points. However, the gradient at this point more than compensates for the greater variation, as can be demonstrated by transferring the deviation, here shown as vertical lines through the mean points, to the abscissa.

Although the highest accuracy is obtainable by determining the exact amount of hormone which will produce this or some other arbitrarily chosen

TABLE 1. CHANGES IN UTERINE TISSUE WATER FOLLOWING ESTROGENIC STIMULATION (ESTRADIOL)[1]

Hours after 0.1γ single dosage	0	2	4	5	6	8	12
% water	80.9	81.5	84.8	85.3	85.8	84.3	83.3
Dosage in gamma	0	0.006	0.0125	0.025	0.05	0.1	0.2
% water at 6 hours	80.9	80.7	82.2	84.2	85.4	85.8	85.9

[1] Averages of 42 determinations on 290 animals.

degree of uterine response, accurate results can also be obtained by reading from the standard curve from any point within the reactive range. This may be illustrated by the preliminary assays on estrone. Four groups of 5 to 7 animals each were injected with the following dosages 0.25 γ, 0.33 γ, 0.5 γ, and 1.0 γ of estrone; the uterine weight increases were respectively 28.7 per cent, 37.1 per cent, 47.2 per cent, and 61.5 per cent. Reading off the values from each of these individual points which included the extremes of the curve, the equivalents of 1 γ estradiol in γ of estrone were 12.7, 12.2, 11.4, 13.6, the average 12.4. The actual figure as obtained by this test is 12.0 γ.

As shown in figure 4, the maximal dose in the reactive range (0.1 γ) is 16 times greater than the minimum (0.006 γ). This wide range of dosage over which the uterine response is proportional greatly facilitates assay, for the number of dilutions of an unknown solution which must be tested need be very few. For example, if an unknown solution be diluted in multiples of 4, and each dilution injected into a group of 5 animals, at least 2 of these dosages will fall within the reaction range, and from them the estrogenic potency can be read directly. A very close approximation to the true value can thus be obtained in 6 hours. Greater accuracy can then be achieved by injecting a large group of animals with the previously estimated unit.

It is of course important that assays be carried out with reference to a standard. In these studies, synthetic estradiol was used, but it has subsequently been found that estrone serves equally well as a standard of reference, for the two substances are qualitatively identical by this test, and give a quantitative

difference of 1:12. It is recommended that a standard curve first be constructed, using the strain of rats which are subsequently to be the assay animals, and that assay results be read from this curve in any convenient units. The following equivalents have been found to hold, and the abscissa of the curve may be expressed in any unit which is most convenient: 1 γ estradiol ≡ 12 γ estrone ≡ 12 Allen-Doisy units ≡ 120 I.U. ≡ 40 U as here described. These results are in exact agreement with the figures obtained by Whitman, Winstersteiner, and Schwenk (2) for estrone and estradiol, using the vaginal smear technic in the adult castrated rat.

DISCUSSION

Evidence from a number of sources indicates that injected estrogen is rapidly mobilized and promptly active. In the rabbit, Markee (3) noted a cessation of the vascular rhythm of endometrial ocular transplants 20 minutes after a subcutaneous injection of estrogen. Allen, Smith and Gardner (4) found that estrogen injections in mice were closely followed by mitotic activity in the vagina; the uterus was similarly affected a little later. They noted an edematous condition of the endometrial stroma 9½ hours after injection, at the time when mitotic activity was just beginning.

From a physiological point of view, the early accumulation of water in the uterus appears to be significant, as it precedes mitotic activity. At the time when mitoses are prominent in the uterine epithelium, the accumulated water is already subsiding, and has returned almost to normal by the time of the great multiplication of cells between the 24th to 48th hours. This cellular division may possibly be correlated with the second weight gain mentioned above. It is felt that this imbibition of water may be a means by which the hormonal effect on the end organ is primarily mediated, and continued investigations are being carried out to test this hypothesis.

A number of workers have found that the ovarian hormones influence the water content of the uterus. For example, Hartman (5) described a rapid loss of the 'edemia' of the pregnant opossum endometrium following castration, and Okey, Bloor and Corner (6) showed that the pig uterine mucosa contains more water during the stage of 'congestion'. Kahayyal and Scott (7) found that the uterus of the mouse, but not that of the rat, had a higher water content in estrus. David (8) did not find these differences in the mouse. Van Dyke and Ch'en (9) reported that both mucosa and muscle of the monkey's uterus were higher in water during the stage of follicular ripening, and after estrogen injections in castrates than at any other time.

In the light of the foregoing results the question naturally arises as to whether the water accumulation is secondary to vascular or other changes, or whether it represents a primary direct action of estrogen upon specific tissues and cells. The promptness of the response points to a direct action, but whether the hyperemia is an intermediary effect remains to be determined.

Of the many methods which have been proposed for the assay of estrogens, few need be mentioned here. For reviews of the various technics and their respective merits, the papers of Allen and Doisy (10), Störmer and Westphal (11), Marrian (12) and Schmidt-Thomé (13) may be consulted. Several technics have been developed, using the uterus of the immature rat (14, 15,

16), and the immature rabbit (17). These tests depend on injection periods of 4 to 8 days and probably have no advantages over the Allen-Doisy test; in fact, Bülbring and Burn state that the variation is so great that the rat uterus is unsatisfactory as a test method. Some workers, David and de Jongh (18), Dorfman, Gallagher and Koch (14), claim a differential effect of various estrogens on the vagina and uterus of the rat. This may well be the case with the longer test periods, for whereas vaginal cornification is a self-limited process, growth of the uterus is a gradual summation effect. For the slowly absorbed synthetic hydrocarbons possessing estrogenic activity no accurate assay has yet been developed. These and many other estrogens are yet to be assayed by this new method.

SUMMARY

Certain early effects of estrogen on the uterus of the immature rat have been described. Following a single injection of 0.1 γ estradiol there is a prompt increase in uterine weight which reaches a maximum of 60 per cent in 6 hours followed by a fall. This weight increase is accompanied by, and almost entirely due to, an increase in the water content of the uterus. This water is accumulated chiefly in the stroma of the mucosa, and to a lesser extent in the subserosa and between the muscle layers. Using this 6-hour maximum as a time interval, a standard curve has been constructed relating uterine weight response to estradiol dosage. From 0.006 γ to 0.1 γ estradiol a direct logarithmic relationship has been established between dosage and uterine response. Based upon this, an assay method has been evolved permitting the accurate assay of estrogenic substances in 6 hours. Tests are performed by injecting dilutions of the unknown solution into groups of 21 to 23-day-old rats, and determining the average uterine weights 6 hours later. From the weight increase the estrogenic potency of the solution is read directly from a standard curve. The test has been standardized with estradiol and estrone, so that assay results can be expressed directly in I.U.

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THE MENOPAUSE

A CONSIDERATION OF THE SYMPTOMS, ETIOLOGY, AND TREATMENT BY MEANS OF ESTROGENS

HANS WIESBADER AND RAPHAEL KURZROK
From the Department of Obstetrics and Gynecology, and the Sloane Hospital for Women, the College of Physicians and Surgeons, Columbia University
NEW YORK CITY

The treatment of menopause symptoms by ovarian extracts dates back a full generation, but therapy by means of pure hormone preparations is of very much more recent origin. Large quantities of pure estrogens have become available only during the past 5 years. This report deals with the treatment of 200 cases of menopause by means of purified or crystalline estrogenic hormones (except one). These patients have been observed over a period of not less than 6 months and, on the average, not more than 3 years years. Several patients have been observed for about 4 years.

Distribution of cases. The etiological factors in the causation of the menopause have been as follows: spontaneous menopause, 160 cases; surgical menopause, 34 cases; radiation menopause, 6 cases.

SYMPTOMATOLOGY

In every case an attempt was made to determine the predominant symptom that brought the patient to the Clinic. Other symptoms were also carefully noted. The symptoms were as follows.

Disturbances of menstruation. Amenorrhea was one of the most common complaints. Twenty-nine patients were still menstruating, but presented other symptoms characteristic of the menopause. Of this group 18 patients reported fairly regular periods, whereas in the remaining the bleeding ranged from oligomenorrhea to hyper-polymenorrhea. It was interesting to note the effect of menstruation (or bleeding) on the symptoms. In 12 cases the symptoms definitely improved with bleeding, in 8 cases the symptoms were made worse, and in the remainder no difference was observed.

Neurovascular disturbances. These were observed in almost all patients to a greater or less degree. The symptoms were hot flushes, sweats, palpitations, sleeplessness, numbness or tingling in the extremities and sensory neuroses especially of the throat. Hypertension appeared rather frequently. In view of the fact that many of the patients were not seen by us prior to treatment, it was very difficult to determine to what extent the hypertension was solely attributable to the menopause. If as a result of 4 weeks treatment there

occurred a persistent drop of 20 mm. in the blood pressure, then we felt that the menopause was a contributing factor in the hypertension.

Severe headache of a migraine type occurred in a number of patients, several of whom had no such headaches previously. In others, who had experienced this symptom at menstrual time, the headache recurred at periodic intervals, even though bleeding was absent. Whether this cyclical phenomenon represents the carrying over the cyclic pituitary function characteristic of the female is an interesting consideration.

Pelvic atrophies. Pruritis vulvae occurred in a number of patients. In some it was a persistent symptom, but in others more or less fleeting in character. These patients, studied as a group, especially in their relationship to the vaginal smear (6), will be reported on in a separate paper by Dr. Irja Widenius.

Disturbances of other glands of internal secretion. In this group were included the cases of definite metabolic dysfunction. A weight increase (20 lb. or more) occurred in about one-third of the patients. There was a general tendency for the fat depots to be localized in either upper girdle, lower girdle or trunk region. The latter group predominated. Another 10 per cent showed joint symptoms. Excessive fatigue was frequently noted. Dysfunctions of the thyroid were fairly frequent, with the hypothyroid state predominating. Only 2 patients showed excessive hair growth, but had no other signs of virilism.

The conclusion should not be drawn that all patients undergoing menopause had distressing symptoms. Only patients presenting definite symptoms were referred to us for therapy.

THERAPEUTIC MEASURES[1]

The following substances were used.

1. *Estrogens.*
   A. For intramuscular use (in oily solution):
      Progynon-B, 1 cc. = 50,000 I.U.
      Amniotin, 1 cc. = 10,000 I.U.
      Theelin, 1 cc. = 10,000 I.U.
   B. For peroral use:
      Amniotin, 1 capsule or tablet = 2,000 I.U.
      Theelol, 1 capsule = 0.06 mg. or 0.12 mg.
      Progynon-DH—1 capsule or tablet = 200 to 600 I.U.
      Emmenin—120 day-oral U per teaspoon.
2. Calcium, either in the form of the lactate (3 to 4 gm. per diem) or gluconate (3 to 4.5 gm. per diem).
3. An alcohol ether extract of the whole ovary (commercially available as Sistomensin).
4. Other specific measures such as sedatives, thyroid extract, iron, etc., where definitely indicated.

The rationale for the use of estrogenic hormones needs no extensive explanation to-day. We feel that the loss of ovarian function at the time of menopause is due to an inherent failure of the ovary to respond to gonadotropic

[1] Amniotin was furnished by E. R. Squibb & Sons, through the courtesy of Doctors H. Sydney Newcomer and John Anderson. Progynon-DH and B was furnished by the Schering Corporation through the courtesy of Dr. Erwin Schwenk. Theelin and theelol were furnished by the Parke Davis Company through the courtesy of Dr. Oliver Kamm. The ether-alcohol extract (Sistomensin) was furnished by the Ciba Corporation through the courtesy of Mr. Robert Mautner. Emmenin was furnished through the courtesy of Ayerst, McKenna and Harrison.

(adeno-hypophyseal) stimulation (1, 2, 3). This loss of responsiveness becomes evident in the fourth decade of life and is progressive from then on to its final termination at the menopause. Other evidence has been offered to show that menopause is not due to a failure either of the anterior pituitary gland or of the individual to respond to estrogenic hormone. In fact, during menopause a greater quantity of gonadotropic hormone is contained in the anterior pituitary gland, and a greater quantity is excreted in the urine than under normal circumstances. Hence gonadotropic hormones are contraindicated as a therapeutic measure for menopause symptoms.

The loss of the functioning ovary (i.e., its hormones) from an oriented chain of glands of internal secretion leads to a disturbance in equilibrium in these glands. The symptoms are the result of this lack of equilibrium, and the consequent dysfunction of the remaining glands of internal secretion and of the autonomic nervous system. The administration of follicular hormone (or its derivatives) tends to readjust this lack of nervous and glandular equilibrium, and thereby alleviate symptoms. Estrogenic therapy is maintained until the individual finally readjusts herself to this loss of ovarian function.

The estrogenic substances were used in the following manner.

During the first month. We have observed that the best therapeutic results were obtained if we began with large doses or estrogens. Fifty thousand I.U. of estrogen was administered twice a week for 4 successive weeks. An interval of at least 3 days between successive doses was found most effective.

After the first month. The same number of units per dose was maintained but the interval between doses was increased until an interval of 12 to 14 days was reached. Once this maximum interval, seemingly consistent with good results, was reached, we began to reduce the dose per injection. The lowest amount eventually injected ranged between 2 and 4000 I.U.

Having reduced the interval to about 2 weeks and reached the minimal injected dose, we attempted to change to mouth medication. This was wholly successful in some of the cases. It required about 600 to 1000 I.U. of either progynon-DH, theelol, amniotin or emmenin once or twice a day to keep the patient symptom-free. In general, there seemed to be little choice between these 4 products, although one occasionally felt that any given patient would do somewhat better on one preparation than on another. There appears to be considerable variation in the extent of absorption of estrogens from the gastro-intestinal tract. What factors influence such absorption are not as yet known. It was found best to administer the medication before meals.

The use of calcium. The rationale for the use of calcium is that it is one of the ions that decreases irritability of cells. In view of the fact that many symptoms of the menopause are due to hyperirritability of the vasomotor system, the use of calcium seems justified. When given alone (without estrone) it will not improve the symptoms to any extent. The dose ranges from 3.0 to 4.5 gm. per diem, by mouth. The same dose is maintained throughout the period of treatment. We have not given calcium intravenously in any of our cases.

Alcohol-ether extract of ovary. The use of such an extract in the treatment of menopause has led to considerable divergence of opinion on the part of the members of our own group. The rationale for its use is not entirely clear. It might be asked whether the ovary produces only ova, estradiol and progesterone, or whether there are other substances produced which have some effect on the metabolic processes of a woman. Surely, the effect of such an extract is not by virtue of its estrone content, for 1 cc. rarely contains more than 1 to 3 R.U. of estrone. Amounts up to 10 cc. fail to give a positive test for progesterone. If used alone without estrone, it rarely alleviates symptoms. On the contrary, when injected together with the estrogens, the effect of the latter seems to be enhanced, at least in some patients. The effect produced is not due to dilution of the estrone by the oil used as a vehicle of the alcohol-ether extract. We have added 1 cc. of this extract to every injected dose of estrogen. When the estrogen was given by mouth, the administration of the alcohol-ether extract was stopped.

The use of other measures. In view of the fact that any organ system may become involved as the result of the hormonal dysbalance occurring at the menopause, other therapeutic adjuvants are often essential. These may be rest, diet, sedatives, thyroid extract, hematynics, etc.

It is not to be expected that our 3 major therapeutic measures will alleviate all symptoms which may arise. That is understandable, for we do not as yet know what mechanism is responsible for the production of the symptoms. Neither the absence of estrone, nor the heightened production of follicle-stimulating hormone, nor both together are responsible for the symptomatology. Such hormonal variations may occur in other conditions without producing menopause symptoms. It is conceivable that the loss of estrin plus the excessive production of follicle-stimulating hormone acting over a period of time may be the trigger mechanism which eventually sets the entire menopause syndrome in motion. This explanation is far from satisfactory, for menopause symptoms may occur when these hormones are present in normal amounts, as in those cases of menopause wherein the symptoms are fully developed and the patient is still menstruating normally. This brings us back to the question whether it is not the loss of some other, not as yet known, ovarian secretion that is responsible for the syndrome of menopause.

We have used sedatives sparingly and in relatively few cases because we did not find them essential, and their continuous use in a prolonged case of menopause may result in habit formation.

In our opinion, all gonadotropic hormones are contraindicated as a therapeutic measure during the menopause. A number of writers are still maintaining that such therapy as we have recommended above functions by virtue of the psychic effect of the doctor upon the patient. They insist that sterile water will produce equally good results. Although we do not underestimate the therapeutic value of suggestion in medicine in general, we do deny that sterile water or any such similar placebo will depress the production of follicle-stimulating hormone by the anterior pituitary gland (4, 5) or change the character of the vaginal smear (6).

RESULTS AND DISCUSSION

The response we have obtained to this form of therapy varied in two types of cases: *a*), patients still menstruating fairly regularly, and *b*), patients with complete amenorrhea (whether the result of spontaneous, surgical or radiation menopause).

We may state at the outset that some measure of relief was obtained in all cases. The best results were obtained in the patients who had stopped menstruating completely. Patients who were menstruating regularly did not respond so well. As stated above, some other unknown factor could be responsible for the production of their symptoms, and our therapy did not replace this last (or disturbed) factor. If we take the reduction in the number of hot flushes and sweat as an index of the effect of therapy (Albright) then the patients who were still menstruating reduced their flushes by two-thirds. On the contrary, a patient with complete menopause frequently reduced her flushes from 40 to 50 per day to 1 to 2. The improvement manifested itself not only in the bettering of isolated symptoms, but there was often a marked improvement in the well-being of the patient. Irritability, sleeplessness and fatigue rapidly subsided.

The arthritic changes arising at the menopause (except those due to focal infection) respond to therapy, but the response is not the same in all patients presenting this symptom. Possibly the patients who responded only moderately to therapy suffered from a type of arthritis that was both infectious and metabolic in character. In general, it required the persistent injection of large doses of estrogens (50,000 I.U.) twice a week, over a period of two or more months, to obtain a good result in the responsive type of patients.

We cannot express an opinion as to the effect of our therapy on cases of involutional melancholia. No such patients were present in our series. But mental depression was complained of by a number of patients. We were careful not to question a patient directly concerning this symptom, for many patients are definitely grieved at the thought that their childbearing years are over (even though children are no longer desired), but that is not mental depression. It was interesting to find that almost all the patients complaining of this symptom previously suffered from premenstrual depression. Such depression as we have observed was definitely relieved in all except one patient. The dosage as outlined above seemed adequate.

Patients suffering from migrainous headache were definitely relieved. Often their usual menstrual headache continued on into the menopause, and maintained a certain rhythmic regularity. In others all rhythmicity was lost. In a third group the headaches made their first appearance at the time of the menopause. The best results were obtained when the estrogens were injected several days preceding the headache. The patient experienced a sensation of impending headache which never materialized. Estrogens injected at the time of the headache had no effect on its duration or intensity.

One disturbing effect of the treatment is the reappearance of uterine bleeding in those cases in which menstruation had previously ceased. Examination of endometrial biopsies obtained at the time of bleeding showed a prolifera-

tive type of endometrium. Cystic hyperplasia was noted in one case. A premenstrual form of endometrium was not found in any biopsy. The total dosage of estrogen which produced the bleeding varied greatly, ranging from 250,000 I.U. to 2,000,000 I.U. All bleeding ceased when the injections were discontinued or the dose decreased. It never recurred spontaneously, but only upon the renewal of injections of large doses (10,000 I.U. or more). Occasionally it required a larger dose to alleviate symptoms than to induce bleeding. The menopause symptoms were entirely relieved in some patients during such bleeding, and made distinctly worse in others. The interval since the last spontaneous period ranged from 4 months to 3 years.

When such bleeding does occur, is it harmful to the patient? May it, if persisted in for a long time, lead to malignant change of the endometrium? No such malignant effect has ever been noted. In our series, whenever bleeding occurred the administration of estrogens was temporarily discontinued and then resumed at a level low enough to prevent recurrence.

The problem of induced bleeding comes to the fore in one interesting group of cases, namely those patients previously radiated for fibroids, whose chief symptom was bleeding. In the few cases we have observed, renewal of the bleeding occurred following the administration of large quantities of estrogens, and promptly ceased when the injections were discontinued. Another question arises at this point. What is the effect of estrogens on existing fibroids (whether symptom-free, or causing bleeding or pressure)? We are unable to answer this question at the present moment. No detectable growth of the fibroids was noted during the progress of therapy, nor was any shrinkage observed. We could not compare our results with other cases of radiated fibroids receiving no estrogens. Although it is known that fibroids shrink in size following termination of ovarian function, exact measurements on the rate of shrinkage were not available.

It appears then that the administration of estrogens for the relief of menopause symptoms may have certain disadvantages. Could the estrogenic hormones be replaced by androgens or by progesterone? Experimental studies have indicated that androgenic preparations are able to limit the secretion of gonadotropic complex by the anterior pituitary (7, 8, 9, 10, 11, 12). It is true that estrone possesses a greater efficiency in limiting the gonadotropic activity of the pituitary than the androgens. The studies of Hertz and Meyer (13) indicate that as little as 0.2 γ of estrone will inhibit the male pituitary as effectively as a daily dose of 30 γ of testosterone or 15 γ of testosterone propionate. They also show that the action of the castrate female pituitary is completely suppressed by testosterone. Furthermore, the effect of progesterone should receive greater consideration. Fels (14) reported that castrated male and female rats developed a typical castrate hypophysis in spite of treatment with progesterone. We are now treating a group of menopause cases with androgens or progesterone (or both). The results will be reported in a separate paper.

Two other questions present themselves, namely, when should treatment begin, and when should it end? Treatment should not begin before definite

symptoms appear. We have begun treatment two weeks after a surgical menopause when the symptoms were as yet insignificant, but we found that it neither lessened the amount or duration of therapy. At the present time we begin when distressing symptoms appear (frequent hot flushes, sweats, insomnia). Similarly, we continue treatment until there is a tendency for distressing symptoms to disappear. This varies according to the patient; it may range from 6 months to 3 or more years. If, some time after treatment has been discontinued, definite symptoms reappear, a short course of therapy is again instituted.

SUMMARY

Two hundred patients were successfully treated for menopause symptoms by means of estrogens, an ether-alcohol extract of the whole ovary and calcium. The best results were obtained in patients in whom the menopause was complete, that is, in whom menstruation had ceased completely. Uterine bleeding (not menstruation) may result from such treatment. The significance of this is discussed.

We are indebted to Professor Benjamin P. Watson for his sympathy, coöperation and assistance in this work, and wish to thank Dr. J. C. Kilroe for his assistance in this work.

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STUDIES RELATING TO TIME OF HUMAN OVULATION

III. DURING LACTATION

PAUL M. LASS, JANE SMELSER AND RAPHAEL KURZROK
From the Departments of Obstetrics and Gynecology and Biochemistry of the College of Physicians and Surgeons, Columbia University, the Sloane Hospital for Women
NEW YORK CITY

It is a well-known clinical fact that, in the absence of any contraceptive measures, some women will become pregnant during their period of lactation, whereas other will not. This has led to the conclusion that ovulation occurs in the former group but not in the latter. A review of literature failed to reveal any statistics on the frequency of ovulation in lactating women. A preliminary report on the frequency of ovulation in lactating women was rendered by us in a previous communication (1). This paper presents some additional data, both in the form of new cases and further information on those previously reported. A subsequent report will consider the frequency of ovulation in lactating women during complete amenorrhea.

A group of patients from the postpartum clinic of the Sloane Hospital were chosen. Each patient was nursing regularly but still having regular periods of catamenia. Endometrial biopsies were taken at 4-week intervals by means of a modified Klingler and Burch (2) suction curette (3). On the basis of this biopsy a diagnosis was made of the phase of the menstrual cycle. The presence of a premenstrual (secretory) endometrium implied that ovulation had taken place, and that we were dealing with an ovulatory cycle. On the contrary, the demonstration of a proliferative (post-menstrual) endometrium within 10 days of the succeeding flow implied an absence of ovulation or an anovulatory cycle. One of us (R. K.) has previously shown (4) that the limits of ovulation during a regular 28-day cycle lie between the 9th and the 21st day of the cycle, with the greatest probability that ovulation will occur between the 12th and the 14th day. The first day of bleeding records the first day of the cycle.

Forty-seven lactating, regularly menstruating women were chosen from about 4000 normal postpartum cases, and 194 biopsies were obtained. Of these cases 2 had 9 consecutive monthly biopsies, 1 had 8, 3 had 7, 7 had 6, 3 had 5, 7 had 4, 16 had 3, and 8 had 2 biopsies. Where possible, the cases were followed up to and beyond weaning.

The technic for obtaining the endometrial biopsy specimen is simple, and with a little practice the procedure can be carried out without the use of local anesthesia and with practically no discomfort to the patient.

Case	Age	Days between delivery and first post-partum period	Biopsy 1	Biopsy 2	Biopsy 3	Biopsy 4	Biopsy 5	Biopsy 6	Biopsy 7	Biopsy 8	Biopsy 9	Weaning
1	34	29	P	P	P	P	P	P	P[1]	P		2 weeks after biopsy 7.
2	27	76	P	P	P	P	P	P[1]	P			1 week after biopsy 6.
4	36	93	P	P[1]	P	P						2 weeks after biopsy 2
6	32	53	P	P	P	P	P	P	P	P[1]	S	2 weeks before biopsy 8.
7	27	80	S	S	S	S[1]	S					1 day after biopsy 3.
8	32	57	S	S	S	S	S					Nursing steadily at time of final biopsy.
13	35	91	P	S	S[1]	P	S	S				1 day after biopsy 3.
15	34	96	P	P	P	S	S	S[1]				4 weeks after biopsy 6.
16	20	28	P	S	P	S						Nursing steadily at time of last biopsy.
24	26	60	P	S	P	P	S	P	P	P[1]		2 weeks before biopsy 8 .
36	28	40	P	P	P	P	S[1]	P				3 weeks after biopsy 5.
39	22	100	P	S	P	P	P	P[1]				4 weeks before biopsy 6.
46	26	44	P	C+GH	P[1]							3 weeks after biopsy 3.
27	25	47	S	C+GH[1]	S							2 weeks after biopsy 2.
47	22	47	P	S	S	S	S[1]	S				1 day after biopsy 5.
34	27	74	P	S	Decidua[1]	Decidua						1 week after biopsy 3.
44	36	45	P	P	P	S	S[1]	Decidua	S			2 weeks after biopsy 5.
26	31	122	S	S[1]	Decidua							3 weeks after biopsy 2.
18	25	34	P	P	P	P	S	S	P[1]			3 weeks after biopsy 7.

P = Proliferative or postmenstrual endometrium.
S = Secretory or premenstrual endometrium.

C + GH = Cystic glandular hyperlasia of the endometrium.
[1] — Weaning.

With the patient in the lithotomy position, the cervix is exposed and wiped clean with sterile cotton pledgets. The external os is then touched with a 2% tincture of iodine solution. A graduated uterine sound is introduced to determine the axis of the uterine body and the depth of the cavity. The sound is then withdrawn and the suction curette gently inserted into the uterus. The trocar is removed and a 10-cc. luer-lock syringe connected through the intermediary of a three-way adaptor. In no case did we find it necessary to dilate the cervix.

The specimen is obtained by gentle curettage of the anterior and lateral walls of the uterus, and the material recovered is drawn back into the lumen of the curette by the suction syringe. The tissue is then preserved in formalin, fixed, cut and stained, then mounted in the usual method.

The spotting that occasionally follows a biopsy is always mild, and rarely lasts more than a few hours. To date we have obtained more than 1500 biopsies with only one slight accident. In no case of the present series did we cause the patient any discomfort or abnormal reaction. In 3 patients, an early pregnancy was diagnosed when the biopsy was examined. The removal of a fragment of decidua did not produce miscarriage.

CASES

Case 1 was followed through 8 consecutive monthly biopsies. All biopsies were post-menstrual, therefore all cycles were anovulatory. This patient nursed her child regularly every 4 hours, up to time of the last visit.

Case 2 was followed through 7 consecutive biopsies. All were post-menstrual (anovulatory). She weaned her child 1 week after the sixth biopsy.

Case 4. Six consecutive biopsies were made; the patient weaned her child on the day following the third biopsy. The first and fourth biopsies were post-menstrual; all other succeeding periods were ovulatory.

Case 6. Nine consecutive biopsies were made. The first 8 were post-menstrual. She weaned her child 2 weeks before the eighth biopsy. The final biopsy was ovulatory.

Case 7. Three consecutive monthly biopsies were made. The first was post-menstrual, the second showed cystic and glandular hyperplasia, and the final biopsy was again post-menstrual. She weaned her child 3 weeks after the last biopsy.

Case 8. Three consecutive monthly biopsies were made. The first was secretory, the second showed cystic and glandular hyperplasia, and the third was again secretory. The patient weaned her child 2 weeks after the second biopsy.

Case 13. Six consecutive monthly biopsies were made. The first was post-menstrual, and all others were secretory. The patient weaned her child 1 day after the fifth biopsy.

Case 15. Six consecutive monthly biopsies were made. The first 4 were post-menstrual, the fifth was secretory, and the sixth again post-menstrual. The patient weaned her child 1 week before the sixth biopsy.

Case 16. Six consecutive monthly biopsies were made. The first was post-menstrual, the second was secretory, and the following 4 biopsies were all post-menstrual. The patient weaned her child 4 weeks before the sixth biopsy.

Case 24. The first biopsy was post-menstrual (24 days after the last catamenia, the next biopsy was pre-menstrual (36 days past last catamenia), the third biopsy showed decidua (87 days past last catamenia). She weaned the child 3 weeks

before the fourth biopsy, which also showed decidua. The patient was transferred to the A-P Clinic. She was prematurely delivered at 7½ months, of a normal living child weighing 1430 gm.

Case 36. Seven biopsies were obtained. After the first period, 45 days post-partum, there was no bleeding until 2 days after the fourth biopsy. The fourth and fifth biopsies showed secretory endometrium. The patient weaned her child 2 weeks after the fifth biopsy. The sixth biopsy was taken 28 days after the fifth, and showed decidua. The patient induced abortion with medication and had a 5-day period 12 days after the sixth biopsy. A 'follow-up' biopsy, done 25 days after onset of above period, revealed an early secretory endometrium.

Case 39. Three consecutive monthly biopsies, 122 days between delivery and the first menstrual period, were made. The first and second biopsies were secretory; the third showed decidua. The patient weaned 1 week before the third biopsy. She had only one menstrual period, which occurred 16 days before the first biopsy. (Three months after the last visit she claimed that she had a period and was no longer pregnant. This was probably a case of induced abortion).

Case 46. Four consecutive monthly biopsies were taken, each one to two days before a period. All were post-menstrual. The patient nursed every 4 hours, and weaned the child 2 weeks after the second biopsy.

Case 27. Five consecutive monthly biopsies were made. The patient nursed regularly. All the biopsies were secretory.

Case 47. Five consecutive monthly biopsies were made. The patient nursed regularly, and had regular periods. All of the biopsies were secretory.

Case 34. Six consecutive monthly biopsies were made. The first three were post-menstrual. The fourth, fifth and sixth biopsies were secretory. The patient weaned her child 4 weeks after the sixth biopsy.

Case 44. Four consecutive monthly biopsies were made, with alternating cycles. The first and third were proliferative, the second and fourth secretory. The patient nursed regularly up to the time of the fourth biopsy.

Case 26. Eight consecutive monthly biopsies were made. The patient weaned the child 2 weeks before the ninth biopsy. Six were proliferative and 2 were secretory.

Case 18. Seven consecutive monthly biopsies were made. The first four were post-menstrual, the fifth and sixth were secretory, and the seventh was post-menstrual. The patient weaned the child 3 weeks after the seventh biopsy.

DISCUSSION

The time of appearance of the first ovulatory cycle is irregular. The presence of an ovulatory cycle one month does not predispose to a similar cycle the following month. It is difficult to understand why one patient will show anovulatory cycles throughout, and another fertile cycles, even though the nursing is regular and satisfactory in both cases. Does this imply the inhibition of ovulation by prolactin in one case, but not in the other? Or is ovulation entirely independent of the action of prolactin in the human? According to several investigators, prolactin has a definite effect on the gonads. Riddle and Bates (5) and Bates, Lahr and Riddle (6) found that prolactin caused a rapid and marked decrease in the weight of the gonads of adult birds. Engelhart (7) observed that in rabbits neither ruptured follicles nor corpora lutea were to be found, but instead there were many atretic and primordial follicles and a diffuse luteinization of the whole ovary. Selye, Har-

low and McKeown (8) and Selye, Collip and Thompson (9) injected estrin into lactating rats, and inhibited the appearance of estrus. They considered the inhibition to be due to the presence of large corpora lutea. Lahr and Riddle (10) injected prolactin into adult non-lactating rats and observed the suspension of 2 to 4 estrous cycles, depending on the size of the dose. They found large undegenerated corpora lutea in the ovaries, but the injection of progesterone produced no such suspension of cycles. The character of the cycle does not determine the time of weaning. The cycle following weaning may either be fertile or infertile. The appearanc of a cystic and glandular hyperplasia in 2 cases for a short period is very interesting. It was rapidly converted into a proliferative endometrium in one case, and eventually into a secretory type in the other.

SUMMARY

Forty-seven women studied through their postpartum period had 194 fairly regular cycles. One hundred-and-six cycles (55 per cent) were anovulatory or sterile in character, and the bleeding was not true menstruation since it did not come from a premenstrual endometrium. Eighty-two (45 per cent) cycles were ovulatory in character, hence fertile, and the bleeding represented true menstruation.

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RELATIVE EFFECTIVENESS OF TESTOSTERONE-PROPIONATE AND DIHYDROANDROSTERONE-BENZOATE IN THE CHICK AS INDICATED BY COMB GROWTH[1]

W. R. BRENEMAN

From the Waterman Institute[2]

BLOOMINGTON, INDIANA

It was observed in a previous study (1) that both testosterone-propionate and dihydroandrosterone-benzoate were very effective in producing comb growth in the 5 or 10-day-old chick when given subcutaneously for 5 days. This stimulation of comb growth was accompanied by a temporary cessation of testis growth which lasted through the injection period. After treatment the combs did not retrogress, but continued to grow and to exceed those of control birds. Additional phases of the testis-comb relationship in the normal and experimental chick from hatching to one month of age were investigated in a second paper (2) in which it was noted that the testes were apparently secreting a comb growth-stimulating substance as early as the 10th or 15th day after hatching. It became evident that a more detailed analysis should be made with respect to dosage because, in both of these studies, the injection of testosterone-propionate and dihydroandrosterone-benzoate in the non-caponized chick appeared to give quantitatively similar results on testis weight and comb growth. Likewise, it was felt that the factors concerned with growth and maintenance of comb should be determined as far as possible.

EXPERIMENTAL PROCEDURE

There were 214 single comb White Leghorns used as experimental animals. Control and experimental birds were taken from the same hatch whenever possible, and since other experiments were made at about the same time, for which additional controls were used, about 350 chicks were available for comparison as controls. The two synthetic male hormones employed in these experiments were testosterone-propionate (Oreton) and dihydroandrosterone-benzoate (Oreton-B). The hormones were in oil solution, and dilutions were made with sesame oil; administration was in single or multiple doses and all injections were made subcutaneously. Single injections were made on either the first or the fifth day after hatching, and the multiple injection series were divided into two groups: one in which hormone was injected the 1st, 3rd, 5th, 7th, and 9th days, and the other on the fifth to ninth day inclusive. As in previous work from this laboratory, a comb factor represented by $\sqrt{H \times L}$ mm. was used for purpose of comparison.

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RESULTS

The results presented in table 1 are especially interesting in view of the work of Parkes (3) and Deansley and Parkes (4) on the relative efficiency of the propionic acid ester of testosterone as compared with testosterone. Their results demonstrated that in the capon and castrate mammal the hormone in the form of a 'crystal mush', the propionic ester, or after the addition of certain fatty acids, was more effective. Testosterone propionate when given in a single injection was as efficient as the same amount administered in smaller daily doses. Deansley and Parkes believe that the variations in efficiency of the various preparations studied can be explained best on the basis of differences

TABLE 1. COMB GROWTH IN 10-DAY-OLD CHICKS WHICH RECEIVED TESTOSTERONE-PROPIONATE OR DIHYDROANDROSTERONE-BENZOATE

Series	Treatment Testosterone-Propionate Total Dose mg.	Injections Number	Injections Day	Comb $\sqrt{H \times L}$ mm.	Testis Wt. Average
A (7)[1]	2.5	1 (0.5 cc.)	1 only	6.9	13.5
B (39)	2.5	5 (0.5 cc.)	1, 3, 5, 7, 9	8.8	12.8[2]
C (14)	1.25	1 (0.25 cc.)	5 only	5.9	12.3
D (12)	1.25	5 (0.5 cc.)	5 to 9	7.8	11.9
E (13)	0.625	5 (0.1 cc.)	5 to 9	7.4	13.3
F (13)	0.625	5 (0.05 cc.)	5 to 9	7.4	12.6
	Dihydroandrosterone-benzoate Total Dose mg.				
G (10)	1.25	1 (0.5 cc.)	1 only	8.4	10.3
H (8)	1.25	5 (0.5 cc.)	1, 3, 5, 7, 9	8.6	13.0
J (14)	2.5	5 (1.0 cc.	1, 3, 5, 7, 9	9.1	
K (10)	1.25	5 (0.5 cc.)	5 to 9	8.0	12.3
L (14)	0.625	1 (0.25 cc.)	5 only[3]	7.7	
M (30)	Control	None	10	4.8	17.0

[1] Number of chicks.
[2] Average of only 26 chicks.
[3] About 8 hours over 10 days.

in the rate of absorption, and not primarily on the basis of hydrolysis. When this was checked by injecting the hormones intraperitoneally or intravenously their conclusion seemed to be confirmed.

It is obvious that the injection of testosterone-propionate into the normal (unoperated) chick gave different results than those described by Deansley and Parkes for the capon. Testosterone-propionate was more effective when given in divided doses than in single injections. The injection of a total of 2.5 mg. of the testosterone-propionate on the first day (series *A*) produced combs having an average factor of 6.9 on the 10th day. The same amount of hormone, however, when given in 5 injections of 0.5 mg., each for the same time interval (series *B*), resulted in an average comb size of 8.8. Only 7 birds were used in the first series, but the results are in harmony with the remaining data, which may justify their inclusion with admitted reservation. When the injections were made over a shorter period of time, a response similar to that

of the first two series was observed. A single injection of 1.25 mg. of hormone gave a comb factor on the 10th day of 5.9 (series *C*), while the same amount of testosterone-propionate in equal divided doses (series *D*) produced combs averaging 7.8. The 5.9 average is significantly higher than the control of 4.8, so that there is no question of hormone action on the comb. Because the hormone in series *D* was given in twice as much oil (0.5 cc. compared with 0.25 cc.), it is possible that dilution might have been a factor. A consideration of this point was made by administering doses of hormone totaling 0.625 mg. in either 0.5 cc. of oil (series *E*) or in 0.25 cc. of oil (series *F*). The comb factor averages for these series were the same; namely, 7.4, and average testis weights showed nearly the same degree of inhibition, weighing 13.3 mg. and 12.6 mg. respectively. The results indicate that the amount of dilution as used here and under these conditions is of doubtful significance.

Series *E* and *F* afford some suggestion concerning the utilization of the hormones in these experiments. The amount of hormone injected in these two series, 0.625 mg., was only half as much as that given in series *C*, but the comb factors exceeded those of this single injection series by 1.5. The comb factor is a linear representation, and a consideration of volume would make this size difference even more significant. The possibility of leakage of the oil from the injection sites can not be ignored, and might be an important factor in single injection series such as *C*. This possibility was realized and checked, but leakage was negligible except in rare instances. When loss of oil was noted, the chicks were either re-injected or discarded, usually the latter. Furthermore, the single injection series shown in the dihydroandrosterone-benzoate series, in which comb response was marked, indicate that leakage is a questionable factor.

The results which followed treatment with the dihydroandrosterone-benzoate were surprising, inasmuch as comb growth was greater than that observed in the testosterone series. Not only was comb size larger, but single injections also proved to be very effective. A single injection of only 1.25 mg. of the dihydroandrosterone (series *G*) resulted in an average comb factor of 8.4, which was greatly in excess of that in the comparable testosterone-propionate experiment (series *A*). The comb factor of 8.4, furthermore, exceeded that in all of the testosterone-propionate injection groups except one (series *B*). Treatment with 2.5 mg. of dihydroandrosterone (series *J*) divided into five equal injections on the 1st, 3rd, 5th, 7th, and 9th days produced an average comb factor of 9.1, which was the maximum growth encountered in these experiments. The possibility of dilution should be noted again. A total of 1 cc. of oil was used for the injections in series *J*, and this may have influenced the result somewhat. Perhaps the most conclusive evidence regarding dosage and dilution is to be found in the single injection series, in which 0.625 mg. of dihydroandrosterone-benzoate was administered (series *L*). The average comb size in that series was 7.7. This series was about 8 hours over the 10-day limit, and it is unfortunate than an absolute comparison with the other data can not be made. With due allowance for this extra time, however, it is clear that resultant comb growth was significantly higher than in the comparable testosterone-propionate series, only one of which had a markedly higher size

average. The response also compared well with the multiple injection series of the dihydroandrosterone-benzoate experiments.

One final observation may be made. The response of the comb was not proportional to the dosage used. This was noted previously in similar experiments and when pituitary hormone was given simultaneously with male hormone. It was suggested then that there is a rather definite limit beyond which it is not possible to push comb growth in the chick, and that a time factor plays the important part in limiting comb response. This still appears to be the most logical explanation for the discrepancy between response and dosage in these experiments.

If the chicks given male hormone treatment were not killed when injections ceased, the combs which had hypertrophied continued to grow and did not

TABLE 2. COMB GROWTH IN THE UNOPERATED AND CAPONIZED CHICK FOLLOWING CESSATION OF MALE HORMONE INJECTION

Series		Treatment	Comb Growth $\sqrt{H \times L}$ mm. Days of Age 10	15	20	25	30	35	Testis Wt.
N	(10)[1]	0.25 mg. daily 5th through 9th of dihydroandrosterone-benzoate	8.9	—	—	—	—	—	11.7
N^1	(12)	Same	—	14.3	—	—	—	—	20.5
N^2	(10)	Same	—	—	—	—	27.0	—	61.0
N^3	(6)	Same	—	—	—	—	—	30.0	117.4
O	(12)	0.5 mg. daily on 1, 3, 5, 7, 9 of dihydroandrosterone-benzoate	9.1	14.4	19.6	23.8	27.0	30.2	
P	(13)	0.5 mg. daily on 1, 3, 5, 7, 9 of testosterone-propionate	8.8	11.3	17.0	19.3	22.8	27.5	
♀	(10)	1.0 mg. daily 5th through 9th of testosterone-propionate Capons	6.1	11.2	13.8	15.7	18.2	20.2	
	(350)[2]	Controls	4.8	7.0	12.3	17.6	19.7	22.5	

[1] Number of animals.
[2] Total for all controls.

retrogress. This has been reported previously (1), but additional experiments were necessary in order that the data might be interpreted more fully. Two series of chicks were injected on alternate days from the 1st through the 9th day after hatching; one group received testosterone-propionate, the other dihydroandrosterone-benzoate. Individual records were kept of these birds, and comb measurements were made on the 10th, 15th, 20th, 25th, 30th, and 35th days of age. Two additional series were also run, in order that comparisons might be made. Ten birds were caponized when 5 days old, and injections were started at once and made daily through the 9th day. Combs were measured at the same intervals as in the two previous experiments. A fourth group was injected with dihydroandrosterone from the 5th to 9th day, and part of the birds were killed, combs measured, and testes weighed on the 10th, 15th, 30th, and 35th days. This last group, although they received only one fourth as much hormone, can be compared with the capons with respect to duration of the injection period. These experiments were designed to determine, if possible, what part the injected hormone was playing in the comb response of the older bird and what proportion of the growth, if any, was a result of the action of the chicks' own testes. The data are shown in table 2.

The data in table 1 demonstrated that the combs were larger in the series given the dihydroandrosterone-benzoate. The material presented in table 2 shows that after the cessation of injections this dissimilarity in size became even more marked. The net increase in comb average on the 10th day, because of previous injections, was 4.3 greater than the control, by the 15th day the difference was 7.4. The combs of control chicks increase rapidly in size after the 15th day; nevertheless, on the 20th day the difference between injected and control was 7.3. The dissimilarity was slightly less on the 25th day (6.2), but was again increased to 7.3 on the 30th, and to 7.5 on the 35th day. These figures show that rather uniform curve for comb growth existed in the treated chicks which closely paralleled the normal curve. Continued growth was apparent also in the testosterone series, but this was less marked than in the dihydroandrosterone group. The net increases over the controls were 4.0, 4.3, 4.7, 1.7, 3.1, and 5.0 on the successive 5-day intervals. The 25th day was the time of least difference in both groups, and it is possible that the control data are inadequate for this time series because of the methods of sampling. This control determination was based on 18 animals, all of which were in one series; additional animals may prove this comb average to be somewhat high. All the other time series show a marked degree of uniformity, and no other explanation of the observed variations seems justified at present.

Domm (5) has been able to produce larger combs than those reported here by injection of gonadotropic hormone over a period from the 2nd to 18th day in the Brown Leghorn chick. When injections were stopped, the combs decreased in size and in a short time had a normal appearance. This suggests that perhaps one of the fundamental differences between the two experimental results may have been the use of the male hormone in oil solution in my chicks. The hormone in oil having a slow rate of absorption might thus have had an opportunity to act during the entire observation period. A second suggestion was advanced previously, (1 and 2); namely, that a secretion from the animals' own testes was responsible for the continued growth which was observed in similar experiments. The following data in table 2 are considered from these view points.

The experiments in which 0.25 mg. of the dihydroandrosterone was given from the 5th to 9th day (N, N^1, N^2, N^3) had comb averages on the 10th and 15th days which were only slightly smaller than those of the 0.5 mg. series *O*, but on the 30th day the combs averaged the same and were almost identical on the 35th day. If the injected hormone were solely responsible for the comb maintenance it would seem logical to expect a greater difference in the comb response than was observed, because twice as much hormone was used in the one series as in the other. In addition, between the 5th and 35th days body weight had increased 500 per cent, and the injected hormones would have been subjected to increasingly greater dilution in body fluids or tissues. Likewise, it is difficult to imagine an excretory mechanism which would not greatly diminish the hormone in a 25-day period. In view of these points, it is extremely unlikely that the hormone injected on or before the 9th day was effective throughout the entire 25 or 30 days.

A more critical experiment is the one in which the capon was injected

and the comb growth checked by comparison with the previous experiments and with the control. The heaviest dosage of hormone in any series (1 mg. daily of testosterone-propionate for 5 days) was given the chicks which were caponized on the 5th day, and it was administered in a relatively large volume of oil (0.2 cc. daily). The combs in these capons, however, did not equal at any time those in the dihydroandrosterone-benzoate series, and were even of smaller average size than those of the unoperated birds, which received

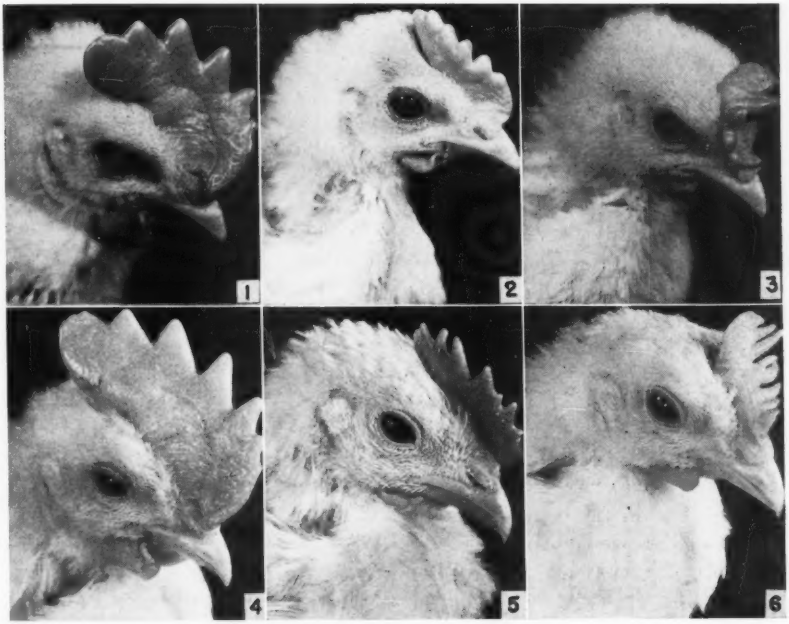

Fig. 1. Chick 25 days old, received 2.5 mg. total dihydroandrosterone-benzoate 1st, 3rd, 5th, 7th, and 9th days.
Fig. 2. Chick 25 days old, received 2.5 mg. total testosterone-propionate 1st, 3rd, 5th, 7th, and 9th days.
Fig. 3. Capon 25 days old, received 5 mg. total testosterone-propionate 5th to 9th day.
Fig. 4. Same chick as No. 1 at 35 days of age.
Fig. 5. Control 35 days old.
Fig. 6. Capon 35 days old, same as No. 3.

only 0.5 mg. of testosterone-propionate daily. The combs of the treated capon exceeded the control averages previous to the 20th day, but after that time the control comb size was greater. It was observed that the combs of the injected capons were less turgid and distinctly less hyperemic on the 20th day, but all growth did not cease at that time. An analysis of body growth (weight) subsequent to the 20th day showed that there was a marked similarity between body weight increase and comb growth. The interpretation of the results of the capon experiment correlates with that based on the injection series in which the intact animal was used, and the conclusion seems apparent. Male hormones which were injected previous to the 10th day were not available during the later time intervals, and it is obvious that the efficacy of the hormones

in the unoperated birds was greater and, therefore, their action was modified by some additional factor.

The photographs illustrate some of the characteristic differences between the two unoperated series and the capons. The bird illustrated in figure 3 shows that the capon comb has already passed the maximum by the 25th day. The comb is no longer erect, and it is less hyperemic than it was previously. The pictures of the chicks at 35 days of age demonstrate the change which occurred between the 25th and 35th days. That the combs of the birds which received dihydroandrosterone continued to grow is quite evident; and although slightly less hyperemic, they remained quite turgid. The comb of the 35-day-old capon indicates that although there has been some increase in length and height, there has been a decrease in thickness, the serrations are more marked, and the comb is also less hyperemic. Other secondary sex characters are of interest. The wattles develop, as shown in the photographs, and the spurs also show a definite increase in size. Perhaps the most interesting response was crowing, which began in some of the dihydroandrosterone treated chicks as early as the 11th day, or only one week after the first injection. By the 25th day nearly all of the treated birds were rather proficient at crowing. The notable exception was that this ability was not observed in any of the capons. Domm also noted crowing in his chicks, but as early as the ninth day of age.

DISCUSSION

Kochakian (6) gave a very excellent review of the literature with respect to the action of the various compounds of testosterone and androsterone, and made a very careful study of the relative effectiveness of a number of them, using the castrate rat for assay. His results indicated the existence of some specific differences between the capon and the rat with respect to hydrolysis of some of the compounds. Kochakian, in disagreement with Parkes, observed that when the interval between injections was increased, the efficacy of the testosterone-propionate was decreased. He is inclined to believe that the hydrolysis of the hormone is quite important. If only the testosterone-propionate series are considered, my data are essentially in accord with those of Kochakian with respect to the greater efficacy of divided injections. This does not hold with respect to the dihydroandrosterone-benzoate experiments. The results of both Parkes and Kochakian are in agreement concerning the fact that in the castrate the testosterone-propionate is the most effective of the androgens tested. Similarily, Koch (7), in tabulating the relative potencies of various androgenic compounds, showed that the testosterone compounds were more effective than the androsterone. It is obvious, therefore, that the unoperated White Leghorn chick reacts quite differently to at least two androgenic substances than does the capon and the castrate rat.

The theoretical aspects of this problem are stimulating. Greenwood, Blyth, and Callow (8) called attention to the fact that capons two years old, which were really incomplete castrates, had larger combs than complete capons when each was given comparable amounts of male hormone. This was also noted later in some of my own experiments with younger incomplete castrates. In view of the fact that small fragments of testes influence comb growth and

that the gonads of the baby cockerel are similarly accompanied by larger combs, it is indicated that the same type of response is involved in both cases. Reference to table 2 demonstrates that the greatest difference between the treated capons and the controls occurred on the 15th day. A comparison, however, of the capon with the testosterone series of the same age shows that the combs were about the same average size. Unfortunately, two factors prevent direct comparison; the injection-time interval was not the same, and the effect of hormonal threshold must be weighed. The dosage used in the intact chicks was 0.5 mg. of hormone daily, in the capon it was 1.0 mg. If allowance is made for the two modifying conditions mentioned above, it is evident that there is some additional factor at work in the intact animal which compensates at least for a part of the 0.5 mg. difference in daily dosage of hormone and for the greater growth subsequent to the 15th day.

It is conceivable that sub-threshold or minimum threshold amounts of an androgen might be secreted in the unoperated chick or incomplete capon. A small amount of such an androgen would be important with respect to subsequent hormone injection because the injected material would be acting at threshold level or close to that point, and less of the hormone would be wasted on a conditioning process. Although this explanation could apply to the testosterone-propionate experiments, some additional condition or conditions must be operating in the dihydroandrosterone series because of its greater activity in smaller amounts and effectiveness in single doses. There is a definite difference in the chemical constitution of the molecules of testosterone and androsterone, and there are some observed differences in their effect on the capon. It seems likely on this account that not only may the hormone be acting near the threshold level but that there may be a superimposing of the action of dihydroandrosterone upon that of the testosterone or an androgen from the testis. Such an explanation might account for the greater response observed after dihydroandrosterone injection, and should be subject to experimental check. This work is in progress.

SUMMARY

The injection of dihydroandrosterone-benzoate and testosterone-propionate into the unoperated chick gave results with respect to comb growth which were contrary to those described in the literature for the capon. Generalizations concerning male hormone action which have been based on treatment of capons are inadequate when applied to the response observed in the normal chick. A survey of the results in 214 experimental and approximately 350 control birds showed that dihydroandrosterone-benzoate was more effective in producing comb growth than was testosterone-propionate. Testosterone-propionate elicited greater comb growth when given in a series of smaller doses than when the same amount was given in a single injection. The dihydroandrosterone-benzoate, to the contrary, was surprisingly effective when given in single injections. Comb growth was comparable to that produced by daily injections and was greater than that in most of the testosterone-propionate series. Combs continued to grow after the cessation of injections, and the data indicated that this continued growth was not a result of the retention and slow utilization of the

injected hormone because comb growth was distinctly less in chicks caponized when 5 days old and given male hormone than it was in the unoperated chicks which received injections. It is suggested that an additional factor was involved in the unoperated chick, perhaps the secretion of some androgenic substance. Development of other secondary sex characters was observed, and crowing occurred as early as the 11th day of age.

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PRECOCIOUS MASCULINE BEHAVIOR FOLLOWING ADMINISTRATION OF SYNTHETIC MALE HORMONE SUBSTANCE[1]

JAMES B. HAMILTON

From the Department of Anatomy, Yale University School of Medicine
NEW HAVEN, CONNECTICUT

In humans who had received injections of synthetic male hormone substance there was noted a tendency toward modes of action that could be considered more virile (1, 2). Since, however, these individuals realized they were being injected with an androgenic substance, and observed resultant stimulation of such responses as lowering of the voice, hair growth and penile development and erections, it is possible that such changes in behavior as occurred concomitantly might have been in some large part due to the patients' awareness of the situation.

To differentiate action of male hormone substance upon behavior, as distinct from responses due to knowledge of the injection of androgens, animals were used. In view of the distinguished character of the cock's actions chicks were chosen for observation of any precocious masculine behavior following administration of male hormone substance.

MATERIAL AND RESULTS

Three series totalling 59 chicks were studied at different dates. Each series was divided into a control and an experimental group. Control animals received daily injections of 0.1 cc. of peanut-oil subcutaneously on the lateral wall of the thorax underneath the wing. Experimental animals received a like amount of oil in the same manner, but each injection contained 500 gamma of testosterone propionate.[2] Injections were begun in all chicks on the second day after hatching, and daily observation made for signs of hormonal stimulation (comb and wattle growth) and behavior modification. Body weights were recorded weekly.

Series 1. Fourteen White Leghorn chicks were selected, by a man experienced in handling chicks, as being females on the basis of the appearance of glands in the cloaca. Seven were kept as control, 7 as experimental animals. At autopsy, however, 5 of the injected and 2 of the control animals were seen to have testes; so the determination of sex at hatching was not accepted. Sixty hours after the beginning of injections comb growth was evident by increase in height and width, by distinctness of comb points and by coloring. By 130 hours the comb was 7 mm. in height, with thick distinct comb points

[1] This study was aided in part by a grant received from the Fluid Research Funds of Yale University School of Medicine.
[2] Testosterone propionate, under the trade name of Perandren, was furnished through the kindness of Ciba Company.

and an orange color. By 180 hours the injected animals emitted strident calls in which the sound was a high-pitched monotone. Despite the high canary-like tone there were many indications that the chicks were crowing. While forming this sound, injected animals mimicked the posture and bodily movements of a rooster engaged in crowing (fig. 1). Study of moving pictures, taken of crowing, showed that the head was elevated, the head-tail axis of the body assuming a more vertical position with tail feathers somewhat depressed. The bill was opened and the neck feathers ruffled. At the peak of these movements vocalization occurred. Injected animals were also prone to frequent lifting and flapping of the wings, accompanied by a rising of the chick upon the ends of the phalanges of the feet. Two of the animals evidenced a tendency to peck viciously at objects brought near to them, and occasionally struck at objects

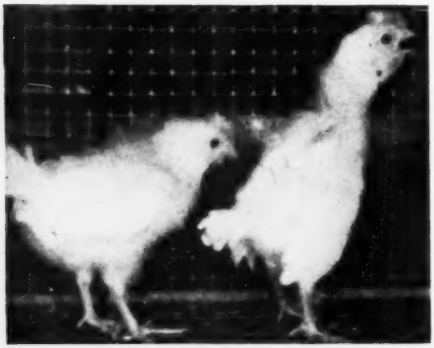

Fig. 1. Enlargement from movie film showing crowing activity in a 15-day-old, 77-gm. chick which had received 13 daily injections of 500 γ of testosterone propionate. Although the crowing and other behavior were those of an adult rooster, the vocal expression was a high canary-like monotone.

with the inner surface of the bones of the feet much in the manner of using spurs, although spurs were not developed by the androgen injections. It is interesting to compare this with the result of Benoit (3) who found by testicular grafts in the cock that crowing, a tendency to fighting and treading might be induced, but that these required more hormone than did changes in plumage, comb and wattles.

The posture of the injected animals was also distinctive. From head to tail the chicks had a foreshortened appearance. The tail feathers were pointed upward. The chest projected anteriorly, while the head was held back on the body to give a question-mark-like appearance of head, neck and thorax. Thus, after 8 days of injection, when the animals were only 10 days post-hatching and weighed but 70 gm. apiece, there were developed cock-like attitudes such as wing-flapping and crowing with appropriate gestures, but without adequate structures such as the syrinx to perform the actions. Movies and sound recordings taken of these precocious actions allowed detailed study of these actions which were proven to be characteristic of the behavior seen in the rooster.

After 27 days of injection the crowing had acquired a diphasic pattern, in that there were at least 3 distinct pitches. Like the call of a cock, the crowing could be modulated from low to high tones and back again. The pitch was lower and the sound was louder, more mature in timber and contained

TABLE 1[1]

Series	Treatment	Animal No.	Sex	Days of Age: 7	14	21	29	39	42	46	49	53	60	63	70	77
1	Control	231	—	80	131	215	—	—	416	—	526	—	—	638	726	816
1	"	233	—	62	98	184	—	—	368	—	459	—	—	568	638	736
1	"	234	—	59	89	166	—	—	369	—	487	—	—	586	696	842
1	"	235	—	78	120	198	—	—	433	—	448	—	—	542	734	852
1	"	236	—	49	79	118	—	—	359	—	—	—	—	—	—	—
1	t.p.[2]	230	—	54	77	114	—	—	186	—	—	—	—	—	—	—
1	"	232	—	70	97	179	—	—	299	—	378	—	—	452	560	657
1	"	237	—	66	89	169	—	—	269	—	348	—	—	420	523	644
1	"	248	—	60	79	154	—	—	282	—	357	—	—	438	502	632
1	"	252	—	66	111	186	—	—	323	—	—	—	—	—	—	—
1	"	261	—	57	83	61	—	—	245	—	341	—	—	372	476	570
1	"	272	—	64	99	175	—	—	293	—	—	—	—	—	—	—
2	Control	50	F	34	63	112	192	438	—	480	—	—	580	—	—	—
2	"	51	"	42	65	145	190	258	—	291	—	—	—	—	—	—
2	"	52	"	44	67	118	176	302	—	347	—	—	412	—	—	—
2	"	53	"	45	80	136	244	396	—	430	—	—	508	—	—	—
2	"	54	"	46	93	156	238	332	—	390	—	—	—	—	—	—
2	"	55	"	53	106	188	302	405	—	453	—	—	—	—	—	—
2	"	176	"	55	95	165	234	362	—	415	—	—	—	—	—	—
2	"	181	"	57	107	176	256	378	—	437	—	—	581	—	—	—
2	"	180	"	42	81	143	226	356	—	405	—	—	500	—	—	—
2	t.p.	182	"	53	93	153	226	315	—	406	481	—	597	—	—	—
2	"	184	"	37	55	87	103	—	—	—	—	—	—	—	—	—
2	"	187	"	49	92	140	180	208	—	361	—	—	—	—	—	—
2	"	189	"	54	90	124	202	327	—	426	—	—	—	—	—	—
2	"	191	"	52	86	124	195	306	—	391	—	463	557	—	—	—
2	"	200	"	32	50	91	137	203	—	279	—	342	447	—	—	—
3	Control	102	F	61	106	192	268	323	—	—	—	—	—	—	—	—
3	"	104	"	49	79	127	149	189	—	—	—	—	—	—	—	—
3	"	105	"	45	88	151	218	253	—	—	—	—	—	—	—	—
3	"	106	"	35	66	130	208	250	—	—	—	—	—	—	—	—
3	"	107	"	52	105	188	—	—	—	—	—	—	—	—	—	—
3	"	108	"	58	101	172	238	285	—	—	—	—	—	—	—	—
3	"	109	"	37	65	117	179	226	—	—	—	—	—	—	—	—
3	"	110	M	50	85	150	—	—	—	—	—	—	—	—	—	—
3	"	111	"	62	109	199	293	346	—	—	—	—	—	—	—	—
3	"	112	"	59	94	160	228	261	—	—	—	—	—	—	—	—
3	"	113	"	45	84	161	248	282	—	—	—	—	—	—	—	—
3	"	114	"	35	73	127	201	264	—	—	—	—	—	—	—	—
3	"	115	"	55	97	173	253	300	—	—	—	—	—	—	—	—
3	t.p.	116	F	36	68	127	—	—	—	—	—	—	—	—	—	—
3	"	117	"	58	108	163	214	264	—	—	—	—	—	—	—	—
3	"	118	"	33	72	124	179	214	—	—	—	—	—	—	—	—
3	"	119	"	38	73	123	194	228	—	—	—	—	—	—	—	—
3	"	129	"	37	68	128	191	250	—	—	—	—	—	—	—	—
3	"	131	"	45	91	152	187	236	—	—	—	—	—	—	—	—
3	"	132	"	31	57	103	161	209	—	—	—	—	—	—	—	—
3	"	133	M	67	100	165	221	278	—	—	—	—	—	—	—	—
3	"	134	"	46	83	139	—	—	—	—	—	—	—	—	—	—
3	"	135	"	47	82	125	186	246	—	—	—	—	—	—	—	—
3	"	136	"	44	70	110	165	237	—	—	—	—	—	—	—	—
3	"	137	"	45	73	134	214	286	—	—	—	—	—	—	—	—
3	"	138	"	48	86	125	196	242	—	—	—	—	—	—	—	—
3	"	139	"	55	92	162	218	270	—	—	—	—	—	—	—	—

[1] The above weights are those of control animals which received 0.1 cc. of peanut oil daily, and of experimental chicks whose daily 0.1 cc. of peanut oil contained 500 gamma of testosterone propionate. These weights are not the maximum possible, for the study was necessarily performed in cramped quarters and in the absence of ideal conditions. Nevertheless, since control and experimental animals were maintained alike, it is considered significant that androgen-injected animals, both males and females, weighed less than control animals.

[2] Testosterone propionate.

less of the canary-like trilling. The combs and wattles were of a great size. The injected chicks had an average weight of 202 gm., the control animals 255 gm. The animals were raised until maturity, but the crowing and wing-flapping characterizing the animals as chicks disappeared as they grew older and were moved at the age of 35 days to outdoor quarters. The injected animals did not develop spurs as did the control male animals, remained lighter in weight (table 1), and were much less resistant to cold weather.

Series 2. To ascertain whether female chicks would assume cock-like attitudes, or whether only the males would respond, 17 White Leghorn chicks were determined to be females by operative inspection. These were injected as in series 1, but although comb and wattle growth was pronounced and weight changes were as in series 1 (table 1), no crowing, wing-flapping or other actions were observed over a period of 55 days despite "no apparent reason why the female (Brown Leghorn) fowl should not crow provided it had the instinct to do so properly developed" (4, 5). It is not impossible that outside factors, such as raising of these chicks in quarters different from those occupied by the chicks in series 1, may have influenced the response to the injected material. It seems definite that certain organs of female animals respond by growth to the presence of androgenic substances. Response in nature is most vigorous and masculine in organs whose homologues in the male are stimulated by androgens (6). This applies particularly to the clitoris (6, 7) and the prostate (8, 9). In fact, the proposal has been made that much of adrenal syndrome as it appears in the human female, including even developmental abnormalities, may be due to the presence of effective amounts of male hormone, and that the symptomatology of the adrenal syndrome may be closely duplicated in female animals by adequate and timely administration of male sex substance (10).

Series 3. Chicks obtained from breeding of Plymouth Rock hens and Rhode Island Red roosters have a patch of white feathers on the head of males, only black feathers on the heads of females. Thus, without operative inspection, selection was made of 6 males and 7 females kept as controls, and 7 males and 8 females which received injections as in series 1. These were kept in the quarters occupied by the chicks in series 2. On the 8th day of injections, when the animals were 10 days old and had an average weight of 68 gm., attempts at crowing were observed in injected males. Crowing activity persisted and, although increasing but slightly in amount during the the following week, was similar in type to that observed in the animals in series 1. After 20 days of injections the experimental animals had an average weight of 133 gm., the control animals 157 gm. (table 1).

SUMMARY

The observations indicate that in the newly hatched chick synthetic male sex substance, although causing stunting of body growth, can stimulate not only gross and cytological growth of organs but also, at least in the males, induction of cock-like behavior. Aggressiveness and crowing appeared in 10-day old chicks weighing 68 to 70 gm. and antedated development of mature, polypitch tones. This stimulation of precocious function is of interest in relation

to the influence of androgenic substances upon sexual and other behavior. The data reported are interpreted as evidence that a behavior recognized as 'masculine' can be induced by synthetic male hormone substance and can be accomplished irrespective of the knowledge of an individual that treatment is being given.

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NASO-GENITAL RELATIONSHIP:

INDUCTION OF PSEUDO-PREGNANCY IN RAT BY NASAL TREATMENT

M. C. SHELESNYAK AND SAMUEL ROSEN[1]
From the Laboratories of the Mount Sinai Hospital
NEW YORK CITY

The existence of a naso-genital relationship has been noted repeatedly in medical literature. Many articles (1, 2, 3) relating to nasal and sexual phenomena have been published, yet the nature of this relationship is vague. Clinical reports revealed nasal changes associated with genital involvements, and conversely genital changes with nasal involvements (4, 5, 6, 7). Experimental investigations are few and limited in scope. Most workers have investigated gonadal degeneration after turbinectomy (8, 9, 10) or nasal mucosa modification after gonadectomy (11, 12). Recently Collip and his co-workers (13, 14) studied the influence of estrogenic substances on nasal mucosa.

To study the nature of the naso-genital association it was necessary to demonstrate, in the laboratory, a measurable reaction in genital behavior to manipulation of the nasal regions; or the converse. In a preliminary study it was shown that the application of silver nitrate solutions to the nasal mucosa induces a modification of the normal and regular periodicity of the estrous cycle (15). Painting or irrigating the nasal mucosa of female rats, having a regular 4 to 5 day estrous cycle, produces a prolonged (12 to 16 days) diestrual phase of the vaginal smear. Examination of the ovaries of animals experiencing this persistent leucocyte phase reveals corpora lutea, and traumatization of the uteri provokes deciduomata. The production of the deciduomata demonstrated that the genital reaction to nasal silver nitrate was pseudopregnancy (16, 17, 18, 19).

The production of pseudo-pregnancy by nasal treatment offered a technic for investigating the naso-genital mechanism. In the previously reported study, silver nitrate solutions were used as the active agent of treatment. Inasmuch as the action of silver nitrate depends on the intensity and extensity of the applied solutions (viz., concentration, volume, persistence, temperature, etc.), and can range from depression of an astringent or a cautery to the stimulation of an irritant, it was uncertain whether the nasal treatment was stimulating or depressing. The experiments reported in this study were designed to ascertain the nature of the nasal tissue reaction which was correlated with the induction of pseudo-pregnancy. Treatment of the nasal mucosa was either by irritant or depressant. Irritation was produced by electrical shocks and by application of oil-of-mustard (*oleum sinapis*). Depression was effected by local precipitation of tissue with protein precipitants and with local anesthetics.

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[1] Technical assistance was contributed by Mr. Philip Rosenberg.

PROCEDURE AND RESULTS

Young adult female rats from an inbred colony were used. The average body weight of the animals at the onset of the experiments was 192 ± 27 gm. The rats were housed in spacious cages and isolated from males. They had a continuous supply of water and were fed a diet of hospital table scraps supplemented with corn and oats, greens, powdered milk, Pablum and cod-liver oil. Smears of the vaginal contents of all stock animals were made daily, and in this way the nature and the regularity of the sexual cycle was determined. Only rats with regular 4 or 5-day cycles were used. No experimental or control procedures were initiated until 4 to 6 consecutive cycles were observed.

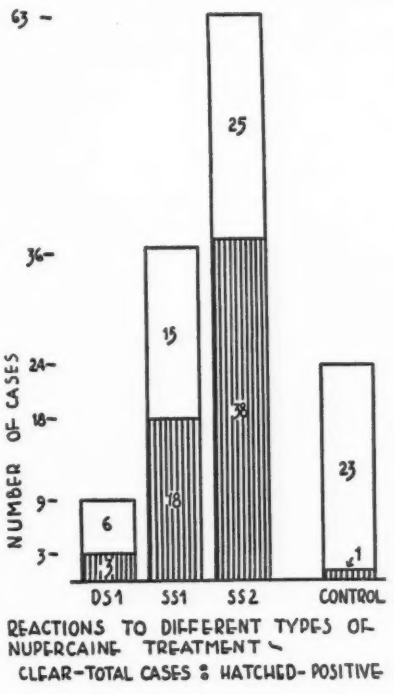

Fig. 1

Except for the series of electrical stimulation all animals were treated with liquid agents. There were two technics of application, *a*), by cotton applicator and, *b*), by irrigation. The small size of the nasal passages in the rat necessitated extremely fine applicators. These were made by winding a wisp of fine cotton around the tip of a dental pulp canal file. Only enough cotton was used to cover the teeth of the file; an excess made the applicator too large. Irrigation was done with modified tuberculin syringes. A 1 cc. tuberculin syringe with a ¼ inch 23 or 24 gauge hypodermic needle was used; an inch-long piece of the finest French catheter was slipped over the needle. The fit was very snug. A spring collar was always attached to the female part of the syringe so that the plunger did not move too freely. Both instruments, the applicator and the irrigator, have semi-flexible tips. This is important because of the hooked or J-like nature of the nasal passage in rats. All treatments, experimental and control, were given while the animals were under light anesthesia. Every effort was made to keep the general anesthesia so light that the animals were ambulatory within one minute after the cessation of treatment.

The time varied in relation to the estrous cycle at which treatment was made. Four types of treatment times were used, *a*), once on the day of proestrus and on the day of estrus (designated 'S' type); *b*), as in *a*), but for 2 consecutive cycles, that is, on the days of proestrus and estrus with rest on the 2 (or 3) days of diestrus, and again once on the days of proestrus and

estrus (designated 'DS' type); and *c*), once daily for 6 days: 2 days of diestrus, day of proestrus, or estrus and two days of diestrus (designated 'SS1'); and *d*), twice daily in the same order as 'SS1' (designated 'SS2').

Treatment of the experimental animals fell into one of two categories: *a*), stimulant; and *b*), depressant. Stimulation was effected by electrical shocks with an inductorium, and by topical application of *oleum sinapis*. Depression was effected by topical application of protein precipitants, tannic acid and trichloracetic acid and by application of a local anesthetic, nupercaine (Ciba).

The special treatment and the results of each series are presented below. Table 1 summarizes all of the data.

Stimulation, electrical. Twelve cases were treated. Bipolar electrodes were passed through the external nares up the nasal passage to the turbinates. The

TABLE 1. SHOWING DETAILS OF TREATMENT AND RESULTS OF NUPERCAINE-TREATED GROUP

Series	Type (See Text)	No. Cases	Body wt. gm.	Total Estrous Cycles	Mean Length Cycles Days	RESULTS: No. Prolonged Cycles	RESULTS: Length of Prolonged Cycles: Mean Days	RESULTS: Length of Prolonged Cycles: Range	Remarks
EXPERIMENTAL GROUP: Nasal Irrigation with 2% Nupercaine									
KAc	'SS1'	10	—	87	4.2	6	13.8	12–16	
1a	'SS1'	16	224	203	4.4	9	11.0	8–15	
2	'SS1'	11	233	67	4.2	2	17.5	13–22	Old animals
1b	'DS1'	15	213	189	4.4	3	13.5	9–18	
3	'SS2'	25	174	143	4.3	20	12.7	8–20	12 gross deciduomata
4	'SS2'	25	216	222	4.3	11	13.3	10–17	
6	'SS2'	13	174	81	4.3	7	12.6	10–21	
CONTROL GROUP: Buccal Irrigation with 2% Nupercaine									
A	'SS1'	14	227	100	4.2	0	—	—	
5	'SS2'	10	205	69	4.2	1	9	—	

electrodes consisted of two dental nerve needles which were insulated with glyptl. Only the tip, 1 or 2 mm. in length, was unshielded. The base of the needles were spot welded to copper leads which were attached to the secondary coil of a small laboratory model (Cambridge) inductorium. The primary coil was in series with two dry cells (6-volt). Shocks were full strength, that is, primary and secondary coils were coaxial and coplanar. Stimulation lasted for one minute. Treatment was type 'S', once on the day of pro-estrus and once on the day of estrus. Ten cases showed no modification of estrus cycles. Two animals had prolonged phase of leucocyte cells in vaginal smears (13 day estrous period).[2]

Stimulation, oleum sinapis. Thirty-nine animals were given topical applications of *oleum sinapis*. All animals received type 'SS1' treatment,—daily applications for 6 consecutive days. In 13 cases the mustard oil was introduced

[2] Harris (personal communication and 20) reported success in producing pseudo-pregnancy in rats by electrical shocking of pituitary and peripituitary regions. Inasmuch as the nasal electrodes could spread afield into the peripituitary regions this type of stimulation was discontinued.

with cotton applicators; in 26 cases by irrigation with 0.05 cc. The oil was in all cases undiluted.

Nineteen animals showed modified estrous cycles after treatment with mustard oil. Three of this group of 19 rats experienced prolonged di-estrual phases (10, 13 and 14-day estrus cycles). Sixteen cases showed disturbances of the cornified cell phase (estrus), and 9 of these 16 had an extra day of estrus (cornified cell smear) during treatment period. Five cases had a number of extra days of vaginal cornification, e.g., 1 case of 5 consecutive days with cornified cell smears.

Depressant, tannic acid. Five animals were given 10 per cent tannic acid applications with cotton tampon. Treatment was maintained for 6 days ('SS').

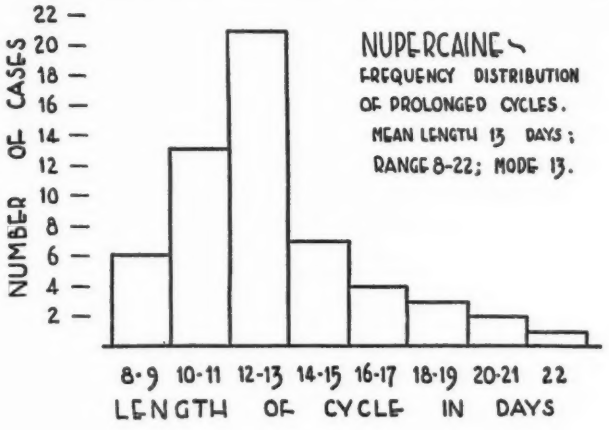

Fig. 2

Four animals showed a prolonged di-estrual phase after this treatment (6, 7, 8 and 14 days).

Depressant, trichloracetic acid. Of 15 rats which received nasal irrigations with 0.05 cc. of 10 per cent trichloracetic acid, 5 had prolonged di-estrual phases.

Depressant, nupercaine (Ciba). In this series of 138 rats, the nasal mucosa of all the animals was irrigated with 0.02 to 0.05 cc. of a freshly prepared 2 per cent nupercaine[3] in 0.9 per cent saline. Nupercaine solutions were prepared from tablets. Fresh solutions were made every other day, so it was not necessary to add any preservative (diluted acid).

The treatment administered to the animals in this group varied in respect to the time of application of nupercaine. Fifteen rats were treated once daily during pro-estrus and estrus on two successive cycles ('DS' type); 36 animals once daily for 6 consecutive days ('SS1' type); and 63 twice daily for 6 days ('SS2' type). Animals in this twice-daily group were given irrigations at 10 a.m. and at 4 p.m. Twenty-four control animals completed the series of 138 animals. Of the 24 controls, 14 were treated once daily for 6 days ('SS1' type)

[3] Nupercaine is a quinoline derivative, and not related to the cocaine or novocaine group; it is the hydrochloride of diethyl-diethylene-diamide of alpha butyl, oxycinchoninic acid.

and 10 twice daily ('SS2' type). Control treatment consisted in nupercaine irrigations of the buccal cavity under anesthesia.

Fifty-eight animals of the 115, which received nasal administration of nupercaine, experienced prolonged cycles following treatment. The long cycles averaged 12.8 $\pm$ 1.2 days, and ranged from 8 to 22 days. The reaction was not uniformly distributed among the different series. One series, the least responsive ('DS') had but 3 pseudo-pregnant reactions in 15 cases. The greatest response was shown in *group 3* of series 'SS2' where 20 of 25 animals became pseudo-pregnant after nupercaine treatment. One of the 24 rats which received control treatment of buccal nupercaine had a 9-day cycle. No other animal in this series experienced any alteration of the rhythmic periodicity of its sexual cycle (table 1).

In order to test the nature of this prolonged leucocyte phase, 16 animals of the nupercaine treated group which experienced long cycles were selected as test cases. On the fourth day of leucocytes the animals were laparotomized and their uteri were traumatized. Silk threads were passed longitudinally through the uteri (21). On the fourth day following the laparotomy, the uteri were examined for decidual growths. All the traumatized sections were prepared for histological examination, and gross observations were checked. Twelve large deciduomatas were found. Microscopic examination corroborated the gross diagnoses.

DISCUSSION

The nature of the naso-genital relationship has not been extensively investigated. The production of pseudo-pregnancy in the rat by nasal treatment with silver nitrate solutions was a manifestation of this relationship, but it offered no clue as to its nature. This was true chiefly because there was no exact ascertainment of the character of the nasal tissue response to the silver salt. Exploration of the mechanism necessitated investigating the general type of nasal tissue reaction which would result in a prolonged di-estrual phase. The simulation of the stimulant and depressant action of silver nitrate was attempted. Irritant stimulation of *oleum sinapis* simulated the irritant action of silver nitrate. This agent stimulates nerve endings in the mucosa which reflexly effect vaso-dilation of the treated regions. The general reaction of the sexual cycle to the mustard oil treatment was not that common to silver nitrate. A disturbance in the periodicity was caused, but this was chiefly of the follicular or cornified cell stage, where the silver salt reaction was associated with a persistent luteal phase. This irritant response of cornified type, coupled with the luteal response to depression, suggests an association of stimulation of the nasal region with the follicular phase. However, critical experimental evidence is not available at present.

Depression by silver nitrate is effected by virtue of complete blockage of tissue. The protein precipitants, tannic and trichloracetic acid were employed to form a layer of 'blocked' tissue. Thus nervous, vascular, lymphatic and secretory function was excluded from the region treated. The fact that a fair percentage of cases treated with the protein precipitants experienced a prolonged luteal phase, suggested depression of nasal tissue as the factor involved.

Specific depression of nervous elements was the first step in determining

the portion of the mucosa concerned in the mechanism. A topical anesthetic was employed. Because of its low toxicity, penetration power and especially its persistent effectiveness, nupercaine (Ciba) was chosen (22). In general, the use of the anesthetic was as effective as the silver nitrate in eliciting long cycles. In certain instances like those where anesthesia was maintained with greater persistence by two daily applications, the reaction of the genital system was more effective ('SS2' *group 3,* 80 per cent). This demonstrates a nervous receptor factor in the naso-genital mechanism. The production of deciduomata in animals with prolonged leucocyte phases verifies the existence of functional corpora lutea and of a progestional endometrium (16, 17, 18, 21). The findings in this study reveal that nervous sensory depression of the nasal mucosa in the rat elicites a state of pseudo pregnancy.

SUMMARY

The periodicity of the estrus cycles of 209 young adult female rats was observed. The effect of treatment of the nasal mucosa with various agents on the estrus cycles was studied in 185 rats with histories of regularity. Control animals received treatment orally. Stimulation with *oleum sinapis* appeared to affect the regularity of the cycles, chiefly in the follicular phase. Depression effected with tannic and trichloracetic acid produced prolongation of di-estrual phase in 9 of 20 cases. Anesthetization of nasal mucosa with nupercaine produced pseudo-pregnant state in 58 of 115 cases. Controls which received buccal treatment were negative. Traumatization of the uteri of 16 nupercaine treated animals with prolonged cycles provoked gross decidual growths in 12 cases.

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A STUDY OF THE RATIO OF THE AMOUNT OF THEELIN PRODUCING UTERINE AND VAGINAL ESTRUS

A. J. SZARKA AND G. KURTZ

From the Laboratory of the Department of Obstetrics and Gynecology of the Royal Hungarian University of Budapest

BUDAPEST, HUNGARY

It is well known that in the different parts of the genital tract the reaction threshold of estrin varies. The amount of estrin capable of bringing about definite growth and cornification on the atrophied vaginal epithelium of a spayed animal is not enough to produce the uterine changes of estrus. Allen (1) states, "The uterine tissues may begin growth, as evidenced by a fairly high incidence of dividing cells, but secretion of the uterine glands may fail to accumulate in amounts sufficient to distend the organ to its typical estrus condition." Brouha and Simonnet (2), Marrian and Parkes (3), and Clauberg (4) also investigated this question. Marrian and Parkes found that, to produce the characteristic estrus distention of the uterus in castrated mice, 200 times the vaginal unit is required. Clauberg successfully produced changes in the uterus of the mouse corresponding to the physiological proliferative phase with only 10 times the vaginal unit.

In the course of our experiments with rats, we tried to find answers to the following problems. *a*), what ratio exists between the amount of estrin required to produce vaginal and uterine estrus, *b*), what is the reaction time curve, i.e., the curve showing the time during which the estrus changes fully develop and disappear.

EXPERIMENTAL METHODS AND RESULTS

One of us (A.J.S.) has already made a brief preliminary report (5) to the effect that more accurate data can be obtained if the development of the hormone effect and the disappearance of these changes are observed upon one and the same animal. For this reason, instead of killing the animal we inspected the uterus, from time to time, through an incision in the abdomen. The laparotomy is performed under ether anesthesia. An aperture of 8 to 10 mm. is made in the abdominal wall 0.5 cm. above the genital papilla, and the intestines are pushed aside so that the uterus is brought into view, first one horn then the other. Following inspection, the opening is closed by clamping together the peritonium and all the layers of the abdominal wall with Michel-clamps. After considerable practice, the operation can be performed in 2 or 3 minutes. Considering the fact that only a macroscopic examination is made, the whole performance does not take longer, and in fact entails even a shorter time than the usual examination of a vaginal smear. Even after much experience, the secur-

ing of vaginal fluid, the preparation of a smear, the staining and the microscopic examination require more time than the inspection of the uterus by means of a laparotomy. Another laparotomy can be performed 6, 12, or 24 hours after the first one.

The healing of the wound in such an initial stage makes it possible to reopen the abdomen without detriment to the final complete healing. In a series of experiments in which we laparotomized the same rats 48, 62, and 72 hours following the first injection, the wound healed completely. This method of inspection through laparotomy can be employed even on a large scale with very little loss. We have animals in our colony upon whom laparotomies were performed 8 times in half a year, and the animals survived without suffering an undue loss in weight.

For the experiment we used rats of our own colony in perfect condition, inbred since 1929, and kept constantly on the same diet. Their weight was also a consideration, as most investigators performing the Allen-Doisy test will agree. In the 116 animals used for examination of the effect of 4.8 γ of ketohydroxyestrin, the body weight varied between 130 and 170 gm. It is advisable that animals used for observation of the uterus reaction should not be used again for the same purpose until a lapse of at least 4 weeks; they should have completely recovered from the previous laparotomy. In order to maintain the required sensitivity of the uterus, no more than 2 weeks should elapse between the examinations of the uterine reaction. For this purpose it is advisable to treat the animal 2 weeks later with the required amount of hormone to produce the uterus reaction, naturally without performing another laparotomy. Of course these animals could also be used in the meantime for the Allen-Doisy test. It must be noted, however, that to maintain the sensitivity of the uterine reaction, at least 4 to 5 vaginal units are required, because one vaginal unit can cause only an initial reaction in the uterus, that is, in the secretion-producing glands. We always used an oily solution of 0.1 cc. each. The second injection took place about 12 hours, and the third 24 hours after the first.

We prepared a vaginal smear in the usual manner twice a day, beginning with the 45th hour after the first injection, and never failed to prepare a smear immediately preceding or following the laparotomy. In some instances we used international standard ketohydroxyestrin, in others a theelin preparation corresponding chemically and biologically to the standard.

In our experiments, we considered 6 different stages in judging the positivity of the uterus reaction. *a*), The uterine horn as a whole increased to 5 mm. in diameter, became filled with fluid, and translucent in its entire length; in other words, it showed the typical picture of what we call 'estrus-uterus'. *b*), On the whole, a similar picture to that above was given, with the exception that the diameter of the uterus was somewhat smaller,—3 to 4 mm. Naturally, the characteristic distention with fluid and glassy translucence were present. *c*), The characteristic distention of the uterus, as described in *a*) and *b*), was only partially present. Hyperemia and swelling could be seen on the parts of the uterus already contracted, but distention with fluid was no longer present. *d*), Accumulation of fluid could be found, if at all, at the tubal end of the uterine horns. For the most part, the uterus was contracted, and only swelling and

hyperemia were evident. *e*), Gross observation betrayed no collection of fluid whatsoever. Hyperemia and swelling were found to a very slight degree. *f*), The uterine reaction was entirely negative, and the organ remained in the thin, thread-like, anemic, castrate condition.

We used these 6 groups during the early stages of our experiments to secure as exact differentiation as possible. However, for practical purposes it seemed better to consider the existence of only 3 groups from the viewpoint of reaction. In judging the vaginal smear, the term 'positive reaction' was assigned to that consisting of cornified cells only.

Under positive-negative signs we assigned those groups in which the vaginal fluid contained only nucleated epithelial cells, or nucleated epithelial cells and cornified cells in clusters, and perhaps 2 or 3 leucocytes per field of vision. We assigned negative-positive signs to those fluids in which, besides mucus and leucocytes, a small number of epithelial cells or cornified cells per field of vision could be seen in somewhat greater number than in the clear diestrus fluid, which was given the negative sign in our examinations.

The effect of a certain quantity of hormone was considered positive if, when administered to a group of at least 15 animals under the above-mentioned experimental conditions, a positive reaction in 70 per cent of the cases resulted, that is, if the vaginal fluid showed only cornified cells and the uterus showed those changes described in the first and second stages, *a*) and *b*).

Our first task was to determine the time required for development of the uterine changes. In our preliminary experiments it seemed that 4.8 γ of international standard ketohydroxyestrin invoked a positive uterine reaction, and for this reason we examined the effect of this amount, performing 150 laparotomies. During the 72 hours following the first injection, we examined the uterus at 8 different times: 14 animals were examined after 36 hours, 38 animals after 44 hours, 19 animals after 50 hours, 14 animals after 52 hours, and 22 animals after 40 hours. Of the 44-hour group we observed 15 animals again 60 hours later; of the 40-hour group we observed 19 animals again 48 hours later; and the 50-hour group we examined again 72 hours later. During these 8 examination periods 150 observations were recorded in 106 animals. The times mentioned for examination were all calculated from the time of the first injection.

From figure 1 it can easily be ascertained that, 36 hours following the first injection of 4.8 γ of ketohydroxyestrin, only 35 per cent gave a positive uterine reaction, but after the next 4 hours we got a positive reaction in 76 per cent of the cases. During the next 4 hours the percentage of positive reactions remained constant, whereas 8 to 12 hours later there was an insignificant rise to 80 or 84 per cent. In the ensuing 10 hours there was a decrease to 31 per cent and 24 hours later, that is, in the 72nd hour, no evidence of a positive reaction.

We also examined the time required for the development of the uterine reaction with twice the above amount of ketohydroxyestrin, 9.6 γ. The rise in the curve up to about the 48th hour gave a result identical with that of the 4.8 γ dosage, and the positive reaction from the 44th to the 50th hour culminated in approximately the same manner. However, the positive-reaction percentage remained at 70 per cent till the 64th hour, instead of falling rapidly, as with

4.8 γ. This was followed by a sudden decline. The only difference with 9.6 γ (double the uterine unit) was that the uterus remained filled with fluid for a longer time. The decline at the end was likewise rapid. This is understandable and to be expected since, if we give a larger amount of estrin, the reaction is protracted and the hormone remains longer in the circulation, thus prolonging the presence of the characteristic estrus-uterus.

During the course of further titrations, we examined the action of different amounts, making our observations at those periods when maximum positivity was expected. Amounts of 2.4 and 4 γ ketohydroxyestrin were tested as regards uterine reaction. Upon examination, 27 per cent of 18 animals treated with

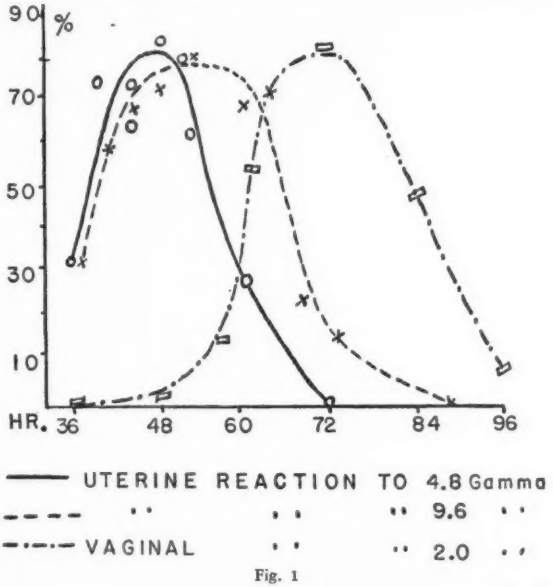

Fig. 1

2.4 γ 48 to 50 hours after the first injection showed a positive result. Of 8 animals examined 54 hours later, 37 per cent showed a positive reaction. Of 38 animals given 3.6 γ and examined after 48 hours, 59 per cent showed a positive reaction. In another group of 38 animals given 3.6 γ and examined 53 hours later, 60 per cent showed a positive reaction. With 4 γ, 53.3 per cent of 15 animals showed a positive reaction, after 50 hours.

These examinations show that 4 to 4.8 γ of ketohydroxyestrin give at least a 70 per cent positive reaction between the 40th and 52nd hours. Hence, this quantity would be the uterine unit of the international standard of ketohydroxyestrin. This uterine unit is 6 times as much in weight as the vaginal unit used in our rat colony to produce the vaginal reaction. The figure is not exact, because the vaginal unit was determined only every 4 to 5 weeks, and the tests for it did not coincide with those for the uterine unit. It is known that the vaginal unit is subject to some change, and we have observed a difference of 10 to 15 per cent in the results.

DISCUSSION

Administering 4.8 γ of ketohydroxyestrin produced changes in the whole length of the genital tract, resembling in every respect those changes seen during the follicular phase of the normal estrus cycle. This leads us to the conclusion that during the physiological estrus cycle some such amounts of estrin are produced in the organism, and that the uterine unit lies closer to the physiological unit than the vaginal unit.

The fact that upon administering hormone the uterine estrus reaction appears sooner than the complete development of the cornified stage is in accord with the sequence of these events during the normal genital cycle. From the

TABLE 1

Protocol	Hr. after injection	No. of rats	Reaction 5 to 6	Reaction 3 to 4	Reaction 1 to 2	% Positive
		Uterine reaction of 4.8 γ theelin				
947 A/5	36	14	—	—	—	35
931/a	40	21	—	5	16	76
604	44	15	—	4	11	75
604/a	44	23	1	7	15	65
931 a	48	19	—	3	16	84
929 c	50	19	1	3	15	80
947/A/j	52	14	—	5	9	64
604	60	15	5	6	4	30
929 c	72	10	9	1	—	0
		Uterine reaction of 9.6 γ ketohydroxyestrin				
981 D	36	19	—	13	6	35
981 B	40	20	—	8	12	60
981 C	44	20	—	6	14	70
981 A	48	23	—	6	17	74
981 C	51	10	—	2	8	80
981 D	60	19	2	4	13	70
981 B	64	19	2	3	14	73
981 C	68	20	12	3	5	25
981 A	72	22	18	—	4	18
938	96	21	—	—	0	0

work of Long and Evans we know that the accumulation of fluid in the uterus begins around the middle of the preestrus stage and reaches the maximum when cornified cells only begin to appear, and in the second half of the cornified stage (third stage of Evans), a distended uterus is practically never seen. The processes of growth in the uterus and in the vagina in all probability begin at the same time, upon the administration of hormone (Allen-Doisy). However, in contrast to the secretion and accumulation of the uterine fluid, a longer time is required for the complete development of the cornified stage.

As to the practical value of the uterine reaction, the following suggestions can be made. By the simultaneous determination of the uterine and vaginal reactions, two figures differing in the ratio of about 1:6 are obtained. If we find in a series of experiments that the uterine reaction in 30 per cent of the cases is negative after 52 hours, whereas the vaginal reaction is positive in all animals, then the true vaginal unit of the hormone examined is surely not less than one-fourth to one-sixth of the amount given. If, however, we find a positive uterine reaction after 52 hours (at which time naturally a complete

vaginal reaction could not yet have developed), it is not necessary to wait 70 to 80 hours, or to make the smear; we can begin with the next dilution of one-fifth or less of this amount, which will show the sought-for vaginal unit. For example, if upon the administration of 100 γ of ketohydroxyestrin, in 3 doses of 0.1 cc. each of oil solution, a positive uterine reaction resulted in 70 per cent of the cases after 50 hours, then it is unnecessary to examine for the vaginal reaction the effects of amounts of more than 20 γ, because the vaginal unit is certainly less than one-fifth of the amount giving a positive uterine reaction (in this case 100 γ). Suppose in 2 groups of animals we gave one 100 and the other 20, and a positive uterine reaction resulted in both cases, naturally the vaginal unit is to be sought for under 4 γ. However, should it happen that the uterine reaction be negative with 20 γ, and the vaginal reaction is still positive, then we know that the vaginal reaction unit is certainly not less than one-fifth of 20, and a dilution of less than 4 is not necessary.

From this it can be seen that 4 to 6 times the amount necessary to produce a vaginal reaction is required to bring about the uterine reaction, following the procedure given. As the vaginal reaction affords a more accurate means of measuring the potency of a certain solution of estrin, the uterine reaction is consequently not employed to displace the vaginal reaction, despite the facts that the uterine reaction is simple, the loss in animals is small, and that results are obtained 20 hours sooner. Unless a much more accurate method is sought, the uterine reaction alone suffices to measure the potency of estrogenic substances. But if it is combined with the vaginal reaction, and the two tests made about simultaneously, the time required for the titration of the solutions is considerably shortened.

It is possible that the ratio between the amounts of estrin required to produce vaginal and uterine estrus may vary in different rat colonies. However, it is also possible that this ratio remains constant in one species. In titrating unknown solutions, this ratio must be determined for each colony and must be checked from time to time with a known solution.

It is surprising that Parkes and Marrian used 200 vaginal units to bring about the characteristic estrus-uterus in mice, whereas we obtained the same results by using only 5 to 6 vaginal units. In explanation of this, the fact should be taken into consideration that different kinds of animals were used (mouse and rat). Also, the methods of administering the hormone, and the methods of observations of the effect were different. The results of Clauberg (4) cannot be compared with ours, since in judging the action of the follicular hormone he used different criteria.

SUMMARY

To produce the characteristic uterine reaction of estrus in rats 4.8 γ ketohydroxyestrin is required. Thus in our colony, and with the method described, the uterine unit is 5 to 6 times the vaginal unit.

The maximal positivity of the uterine estrus reaction (the complete distention of the uterine horns with fluid) is reached 44 to 50 hours following the first injection, almost 24 hours sooner than the appearance in the vaginal smear of the non-nucleated cornified cells characteristic of the vaginal estrus.

In the 72nd hour, when the vaginal reaction is at its maximum, the uterine reaction has completely ceased.

Considering the fact that the examination of the uterine reaction through laparotomies is a simple, quick, dependable method, and entails but little loss in animals, it is suitable for the measurement of the potency of an unknown solution of estrin. It is not expected to replace the Allen-Doisy test but, when combined with it, the time required for the measurement is shortened. The biological effect of 4.8 γ of crystalline ketohydroxyestrin seems to be equal to the effect of that quantity of hormone produced in the rat during the normal estrus cycle.

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SKIN TESTS FOR PREGNANCY

PAUL M. LASS, ERNA S. ENDERLE AND
RAPHAEL KURZROK
From the Departments of Obstetrics and Gynecology, and of Medicine, the Sloane Hospital for Women, the College of Physicians and Surgeons, Columbia University
NEW YORK CITY

Since the advent of the Aschheim-Zondek test for pregnancy numerous attempts have been made to simplify it. The various skin tests for pregnancy represent such an attempt. Porges and Pollatschek (1) were the first to suggest the intercutaneous injection of anterior pituitary extract. Erythema was noted in non-pregnant women at the site of injection. Deutsch (2) and Strauss (3) repeated these tests, and concluded that they were neither definite nor reliable. Gilfillen and Gregg (4) suggested that if a pregnant woman had APL substance in her system, she might not be sensitive to its intradermal application, while a non-pregnant woman might give a reaction. For the test, 2 minims of fresh antuitrin-S is injected intradermally in the ventral surface of the forearm. The reaction is read after ½ hour, or after 1 hour if the reaction is slight. The reaction consists of a wheal surrounded by an area of erythema. The latter may measure from 7 to 46 mm. in diameter, and this alone constitutes the positive reaction. Thus, all pregnant women are negative (no reaction), and all non-pregnant individuals give a positive reaction (erythema). A second report by Gilfillen (5) lengthened the time up to which a positive reaction may appear to 3 hours in patients older than 30 years. Other observers (6, 7, 8, 9, 10) failed to substantiate the findings of Gilfillen and Gregg. The importance of the skin test in clinical medicine led us to investigate its possibilities.

PROCEDURE AND RESULTS

The APL substance used in these experiments was antophysin (Winthrop).[1] This is a flocculent powder obtained by alcohol precipitation of human pregnancy urine. Immediately before use the powder is dissolved in distilled water containing a small amount of merthiolate. The solution assayed 1 cc. equivalent to 100 R.U. One-tenth of a cc. (10 R.U.) was injected intradermally. The similar amount of solvent (merthiolated distilled water) was injected as a control. A positive reaction was based on the presence of a central wheal (often showing pseudopods), surrounded by an area of erythema. A positive test would be charted in the following manner: 3—1—+. The first figure indicates the diameter of the zone of erythema in cm., the second figure the diameter of the wheal in cm. The plus sign stands for the presence of

[1] We are indebted to Mr. John Hart of the Winthrop Chemical Company for a generous supply of this material.

pseudopods. A negative test would read: 0—½—0 (no erythema, no pseudopods). Skin tests showing larger reactions than those of the control were considered positive.

To obtain some idea of the accuracy of this test, 108 pregnant patients were injected with the hormone and the control solutions. The period of gestation ranged from the 2nd to the 10th month. About 50 per cent of the cases were in the last trimester of pregnancy. None of the patients was allergic. Eight patients gave a positive reaction, 100 patients gave a negative reaction. The test was therefore wrong in only 8 or 7.4 per cent of 108 patients.

Twenty-five normal women (non-pregnant) were then tested. Nine patients gave a positive reaction and 16 patients gave a negative reaction. According to the test, 16 patients were pregnant, when actually none was pregnant, an error of 64 per cent.

A third group of patients, comprising 100 non-pregnant women, was then tested. All these patients were allergic to one or many substances. Forty-seven patients gave a positive reaction and 53 patients gave a negative reaction. According to the test, 53 patients were pregnant. Actually none was pregnant, an error of 53 per cent.

Twenty-one allergic men were then injected with antophysin. Sixteen patients gave a positive reaction and 5 patients gave a negative reaction. According to the test, 5 men were pregnant, an error of about 25 per cent.

Gruskin (11) has suggested the use of human placental tissue as an antigen, which, when introduced intradermally, causes pseudopod formation at the site of injection in pregnant women, and a failure of pseudopods to appear in the non-pregnant women. A high degree of accuracy is claimed (94 per cent). He cautions that the test should not be made during menstruation, for positive reactions may appear. It should not be performed in persons with endocrine disturbances, or on hypersensitive skins, which might respond to anything. Schwartz (12) confirmed this test, and obtained an accuracy of 94 per cent.

In view of the fact that Gruskin puts great stress on the technic of the injection, we tried to follow it as closely as possible. An area approximately 4 inches square on the volar aspect of the upper arm was cleansed with alcohol and allowed to dry. Using a ½ inch 27 gauge needle on a 1 cc. tuberculin syringe, 0.1 cc. of control solution (amniotic fluid) was injected intradermally in the shape of a superficial round wheal. About 2 inches away, a similar round wheal was raised, using 0.1 cc. of the Gruskin[2] antigen.

The materials, both control and antigen, were injected slowly, and the results read from 3 to 5 minutes later. In some cases, particularly where the skin was loose, one could see more or less immediate irregularities in the outline of the wheal. These were interpreted as 'skin faults' and were not reported. In such cases the test was repeated on the other arm, and if the 'fault' persisted, the patient was dismissed as unsuitable for testing.

All reactions not definitely positive were classified as negative or doubtful.

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[2] We are indebted to Dr. S. S. Newcomer of the E. R. Squibb & Sons, for the supply of the Gruskin antigen.

The greatest difficulty arose in the 'doubtful' tests, for here two equally competent observers could not agree on the reading of the test. In positive cases the wheal shows pseudopods and a slight inflammatory zone about it. In negative cases no such reaction takes place. A positive reaction appears within 10 minutes.

Our results were as follows. Seventy-three pregnant patients were injected with a Gruskin antigen and with a suitable control (amniotic fluid). The pregnancies ranged from 6 weeks to 6 months. Thirty-five patients gave a positive reaction, 26 patients gave a doubtful reaction, and 12 patients gave a negative reaction. According to the test, only 48 per cent of the patients were definitely pregnant. Actually all were pregnant, an error of 52 per cent.

A second group of 15 normal non-pregnant patients was then tested. Three patients gave a positive reaction and 12 patients gave a negative reaction. The error was 20 per cent.

Twenty males were injected. Five gave a positive reaction and 15 gave a negative reaction. This gave an error of 25 per cent. It was interesting to note that the finest positive reactions occurred in males.

DISCUSSION

Both tests had a margin of error far too great to make them even slightly useful. Neither reaction can supplant the Aschheim-Zondek test. Only one interesting fact appeared, namely, that 92 per cent of the pregnant patients failed to give a skin reaction to PU extract. It might be assumed that the body will form an antibody against these pituitary-like hormones, hence no reaction will occur at the site of injection. This implies that the substances which were injected, all large protein molecules, were the same before and after purification. Antophysin is produced from human pregnancy urine by alcohol precipitation, with subsequent washing of the precipitate with ether. Such treatment would tend to change the character of these proteins and alter their antigenic properties. Such an altered antigen would not react with the antibody within the skin, and some inflammatory reaction would result. The most likely explanation of the failure of an inflammatory reaction to occur within the skin as the result of injection of PU extract is that absorption from the skin differs in the pregnant state from the non-pregnant. Such differences of absorption were previously noted when attempts were made to determine the 'tone' of a pregnant patient (vagotonic or sympathicotonic) by the injection of either adrenalin or atropine. The results varied greatly with the mode of injection, the degree of hydration of the tissues and the B.M.R.

SUMMARY

Two hundred and fifty-four patients were tested for pregnancy by means of the Gilfillen-Gregg skin test PU extract. The error in diagnosis ranged from 7 to 64 per cent depending upon the type of patient chosen for the test. One hundred and eight patients were tested by means of the Gruskin skin test for pregnancy (placental antigen). The error in diagnosis ranged

from 20 to 52 per cent, depending upon the type of patient chosen for the test. Hence neither test is reliable, nor of any value in clinical medicine.

We wish to express our appreciation to Professors B. P. Watson and W. W. Palmer for their kind coöperation in putting at our disposal the clinical material at the Sloane and Presbyterian Hospitals.

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THE EFFECT OF THE ANTERIOR PITUITARY-LIKE HORMONE ON SPERMATOGENESIS IN THE HUMAN

H. S. RUBINSTEIN

From the Laboratory for Neuroendocrine Research-Surgical Division of Sinai Hospital

BALTIMORE, MARYLAND

Because of conflicting reports concerning the effect of the anterior pituitary-like hormone on spermatogenesis (1, 2) it appeared timely to examine this question more critically.

For this purpose 6 healthy adult human males were chosen, from each of whom a number of control spermatic specimens were studied. It was found that, after one ejaculation, the sperm count gradually increased through a period of 6 or 7 days, when the number became more or less stationary. The wide variation in spermatozoal output was taken into consideration, and therefore 2 counts were listed for each individual, a 1-day and a 6- or 7-day control, depending on the interval in days since a prior ejaculation.

Each subject was then given 3 intramuscular injections of the water-soluble fraction of pregnancy urine weekly, beginning with 100 R.U. The dosage was quickly increased to 300 and 400 R.U., so that after 2 weeks a total of 1000 R.U. per week was reached. Dosage was maintained at this level throughout the periods of injections, which lasted with different individuals from 5 to 9 weeks. The hormone was given in the form of a glycerin solution of the water-soluble fraction of pregnancy urine which is now marketed under the name of follutein (Squibb) or antuitrin-S (Parke Davis).[1]

Weekly specimens during and after the period of injections were obtained either in condoms or through masturbation, and were studied for volume, number per cc. and total count. The post-injection period varied with each individual from 4 to 7 weeks. Additional studies were made on masturbation specimens for motility, viability and morphology. All volumes were empirically increased by 0.2 cc. to make up for the amount adhering to the container.

RESULTS

Morphology, motility and viability remained essentially unchanged by treatment. Volume, however, was inconstant throughout the entire period of observation, so that comparisons were made only between total counts. These showed changes which, because of temporal variations with each individual, are tabulated separately (tables 1 to 6).

From table 1 it may be seen that an actual increase in number of spermatozoa occurred 7 weeks after the beginning of injections, and relatively high

[1] The author acknowledges with appreciation the coöperation rendered by Dr. J. A. Morrell of E. R. Squibb & Sons Co. in furnishing the follutein used in this study.

counts were maintained as long as injections were continued. The count in this individual returned to normal about 2 weeks after cessation of injections. Table 2 shows a moderate increase in sperm count after the fourth week of treatment. This increase became more marked after the fifth week. The post-injection period shows counts which are all relatively high but which still fall within normal limits. The last count in this period is higher than a 2-day specimen obtained after 3 weeks of injection. Table 3 shows an increased count appearing after 3 weeks of treatment and remaining so for the duration of treatment. All observations during the post-injection period disclose counts which may be considered above normal. Table 4 shows a marked increase in

TABLE 1. WEEKLY TOTAL SPERM COUNTS IN *case 1*.[1] A 33 YR. OLD WHITE MARRIED MALE, BEFORE, DURING AND AFTER INJECTIONS WITH APL SUBSTANCE

Control	Injection period		Post-injection period	
124.8 (1)	1	176.3 (2)	1	313.6 (5)
328.0 (6)	2	265.4 (6)	2	299.2 (5)
	3	38.4 (1)	3	221.2 (7)
	4	298.5 (6)	4	288.9 (10)
	5	174.1 (5)	5	238.1 (2)
	6	163.7 (3)	6	166.4 (1)
	7	384.0 (6)	7	142.8 (3)
	8	168.7 (3)		
	9	212.2 (3)		

[1] In this and the following tables the numbers in parentheses represent the interval in days since a previous ejaculation. The figures to the left of the sperm count represent the end of successive weeks. All counts are in millions.

TABLE 2. WEEKLY TOTAL SPERM COUNTS IN *case 2*[1]

Control	Injection period		Post-injection period	
96.9 (1)	1	237.1 (4)	1	285.1 (4)
360.8 (6)	2	73.2 (1)	2	253.2 (5)
	3	98.9 (2)	3	273.2 (5)
	4	109.8 (1)	4	334.6 (8)
	5	478.4 (6)	5	119.6 (2)
	6	457.5 (6)		
	7	231.1 (3)		

[1] A 33-yr.-old colored married male, before, during and after injections with APL substance.

sperm count after 4 weeks of treatment. This high count is maintained throughout the period of injections and, after remaining high for 3 weeks thereafter, finally comes down to normal level. Table 5 shows an increased count after 4 weeks of injections which remains high for the duration of treatment. The post-injection period is marked by increased counts for 3 weeks, with normal count thereafter. Table 6 discloses an increased count after 6 weeks of injection, which for some unknown reason drops to a normal level for the next 2 weeks. With cessation of injection, the first 2 weeks show relatively high counts, with return to normal after the third week.

DISCUSSION

From these observations we see that the sperm count was increased in every case. However, the time of increase varied, since in one case it occurred after 3 weeks, in 3 cases after 4 weeks, in 1 case after 6 weeks and in 1 case

TABLE 3. TOTAL SPERM COUNTS IN *case 3*[1]

Control	Injection period		Post-injection period	
126.7 (2)	1	110.2 (2)	1	293.8 (4)
336.2 (7)	2	135.4 (2)	2	408.7 (5)
	3	336.0 (6)	3	441.6 (6)
	4	440.8 (5)	4	168.0 (2)
	5	241.8 (3)	5	190.2 (2)

[1] A 24-yr.-old white single male, before, during and after injections with APL substance.

TABLE 4. TOTAL SPERM COUNTS IN *case 4*[1]

Control	Injection period		Post-injection period	
109.8 (1)	1	253.3 (3)	1	324.7 (4)
352.8 (7)	2	66.4 (1)	2	372.9 (3)
	3	117.9 (2)	3	506.9 (5)
	4	692.8 (7)	4	150.9 (2)
	5	482.4 (3)	5	357.8 (9)
	6	154.6 (2)		

[1] A 33-yr.-old colored married male, before, during and after injections with APL substance.

TABLE 5. TOTAL SPERM COUNTS IN *case 5*[1]

Control	Injection period		Post-injection period	
115.2 (1)	1	247.3 (3)	1	380.8 (4)
352.0 (7)	2	275.3 (4)	2	377.6 (4)
	3	261.4 (3)	3	393.6 (4)
	4	455.3 (6)	4	208.4 (3)
	5	462.7 (4)		
	6	380.8 (3)		

[1] A 26-yr.-old white single male, before, during and after injection with APL substance.

TABLE 6. TOTAL SPERM COUNTS IN *case 6*[1]

Control	Injection period		Post-injection period	
117.2 (2)	1	193.2 (2)	1	282.2 (5)
345.2 (6)	2	274.7 (6)	2	250.7 (4)
	3	328.4 (6)	3	259.9 (6)
	4	187.5 (5)	4	282.2 (6)
	5	432.6 (6)		
	6	174.1 (3)		
	7	205.4 (4)		

[1] A 27-yr.-old white married male, before, during and after injections with APL substance.

after 7 weeks. When once increased, the count remained high for the duration of treatment except in one case (table 6). The cause is unknown, unless it was due to a mistaken report of the age of the specimens, or a general unrecognized illness on the part of the patient. That it resulted from prolonged treatment is unlikely, since it did not occur in *cases 1* or *2*.

In some instances, counts less than the maximum control were considered to be above normal since comparisons, wherever possible, were made with specimens of similar age. This shows the importance of considering maximum and minimum limits when studying the efficacy of a treatment.

The increased sperm found in this study corroborates the report of Brosius and Schaffer (1), who claimed to find such an increase in a young adult male suffering from testicular atrophy as a result of mumps. At the same time it does not negate the findings previously reported (2) in a patient who was subjected to this treatment following the surgical operation for undescended testes, since Brosius and Schaffer's patient before his illness had exhibited complete spermatogenesis, whereas the surgically treated patient had never displayed this function. From experiments carried on in this laboratory, it appears that the A.P.L. hormone, in addition to its interstitial tissue stimulating effect, also stimulates the tubular epithelium to increased proliferation. But it does not hasten the maturity of the particular germinal cells; it stimulates proliferation of cells at the stage of maturation in which the testis finds itself. Hence, in the patient previously reported, it is possible that the germinal cells failed to reach complete maturity, and could therefore not proliferate formed spermatozoa.

SUMMARY

Studies bearing upon the effect of spermatogenesis in the human were carried out on 6 healthy adult males. It was found advantageous to determine the variation in sperm counts obtained at different times. Because the count was found to increase gradually through a period of 6 or 7 days following an ejaculation, control counts were listed for 1 and 6 or 7-day specimens. Injections of A.P.L. substance in dosage approximating 1000 R.U. weekly were administered intramuscularly 3 times weekly for a period varying from 5 to 9 weeks. Sperm specimens collected by condom or through masturbation were studied for volume, number per cc., total number, motility, viability and morphology. Since counts limited to a single cc. were found to be unreliable because of variations in volume, total counts were compared with controls.

Morphology, viability and motility showed no changes from the normal. It was found that in all cases the total count was increased above normal after periods of treatment varying from 4 to 7 weeks, remaining high in all cases but one. With cessation of injections the counts continued high in all cases (including the patient in whom a drop had occurred during the treatment) for periods ranging between 2 to 5 weeks, after which they returned to normal ranges. Differences existing between previous reports are discussed.

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STUDIES ON THE MOTILITY OF THE HUMAN UTERUS IN VIVO

A FUNCTIONAL MYOMETRIAL CYCLE

LEO WILSON AND RAPHAEL KURZROK

From the Department of Obstetrics and Gynecology, the College of Physicians and Surgeons, Columbia University and the Sloane Hospital for Women

NEW YORK CITY

The present study was begun in January 1937 primarily to determine the nature of the uterine contractions in functional dysmenorrhea. It was soon realized that to evaluate these findings properly a thorough investigation of the fundamental aspects of human uterine contractility was a necessary preliminary.

The following material was utilized in the study.

No. of Patients		*No. of Tracings*
2	Normal Menses	6
14	Dysmenorrhea	72
3	Amenorrhea	46
6	Oligomenorrhea	29
3	Ovulatory Bleeding	20
1	Anovulatory Bleeding	14
29	Total	187

The number of tracings per patient ranged from 1 to 32, the average being 6.

METHOD

A small sterile rubber balloon, securely tied to a 12 F Keys-Ulzmann cannula, was introduced into the uterine cavity. It was then attached to a large and very sensitive recording kymograph.[1] Fifteen cc. of air was introduced into the system to expand the balloon. The pressure changes within the balloon were conveyed to a column of mercury on which floated a recording pen (1).

No attempt was made to maintain a constant pressure within the balloon, because of the extreme variability of uterine tonus in different individuals and in the same individual during different phases of the menstrual cycle. In most instances the pressure ranged between 40 and 80 mm. of mercury, and caused the patient little or no discomfort.

The average test lasted about 45 minutes. During the first 5 or 10 minutes the recording was disturbed by a falling pressure, which we attribute to a drop in uterine tonus. The time was recorded in minutes. In most cases an endometrial biopsy, to serve as an index of ovarian activity, followed the

[1] We are indebted to Professor C. C. Lieb and M. C. Mulinos for the loan of this very fine instrument. We are also very grateful for their constant encouragement and advice.

test. Intramuscular pituitrin injections were given in many cases after the character of the spontaneous contractions was definitely established.

In this study our primary aim was to record contractility changes in the same patient at weekly or semi-weekly intervals over a long period of time. Consequently, when sex hormone preparations were injected no attempt was made to determine their immediate effect on the myometrium. The injections were given at the end of the test and the results recorded in subsequent tests.

In interpreting the findings of this study it is important to bear in mind certain temporal considerations relating to the menstrual cycle. Failure to adhere to these principles, we feel, has invalidated the conclusions of some investigators. Although it is customary to date the menstrual cycle from the first day of the flow, the onset of menstruation does not inaugurate a new cycle. It merely indicates the beginning of the end of the old cycle. A new cycle begins with the maturation of a primordial follicle in the ovary. This probably occurs about 3 to 5 days after the onset of the period. The maturing follicle secretes estradiol and finally ruptures, usually between the 10th and 14th days. Within 48 hours after ovulation, the corpus luteum has begun to secrete progesterone. The latter is produced at first in small amounts, but the quantity soon increases and is maintained at a high level until within 1 to 3 days of the flow (2), when it declines rapidly. Some progesterone influence probably persists during and, perhaps, for several days after the flow. During the luteal phase the corpus luteum also produces estrogenic hormone. Thus, some progesterone effect may persist into the early part of the follicular phase and some estrogen effect continues throughout the luteal phase. One hormone 'fades' in as the other 'fades' out. In addition, as will be demonstrated later, the myometrium does not reflect the changes in the ovary as promptly as does the endometrium, but lags about a week behind. For these reasons, the progesterone influence is not apparent immediately after ovulation, nor does it cease abruptly after the onset of menstruation.

OBSERVATION

Uterine Contractions During the Normal Menstrual Cycle

Follicular phase. About 4 to 7 days after the onset of the period, small uterine contractions are present. The contractions are rapid (3 to 5 per minute) and the interval between them is short (fig. 1*A*). Pituitrin (10 U. intramuscularly) usually evokes a prompt rise in tonus and an increase in the amplitude and frequency of contraction. During this phase, however, the uterus is occasionally refractory to pituitrin. A small number of cases show a complete absence of spontaneous contractions, but the addition of pituitrin frequently evokes contractions and a rise in tonus. The uterus rarely remains entirely refractory.

Luteal phase. The follicular phase contractions persist with little variation until about the 17th or 20th day of the cycle (ovulation having occurred between the 10th and 14th days), when they assume a markedly different character. There is some increase in amplitude, but the significant change is that the contractions are now longer in duration (1 or 2 minutes) and the interval

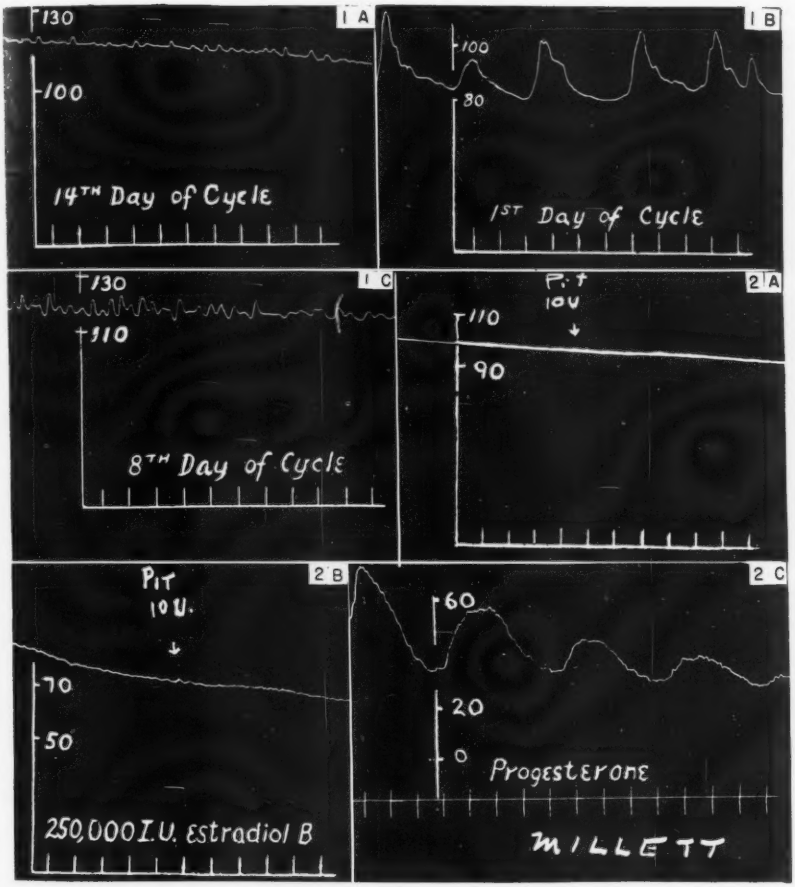

Fig. 1*A*. Patient *A.B.*, 14th day of cycle. Contractions are small, rapid, and of high tonus. Time in minutes.
B. First day of next period. Contractions of large amplitude, slow, and of low tonus.
C. Eighth day of next cycle. Contractions becoming small again, of high tonus, rapid.
Fig. 2*A*. Patient *M.M.* Primary amenorrhea. Before treatment. Complete absence of contractions. No response to pituitrin.
B. After injection of 250,000 I.U. estradiol benzoate during 2½ weeks. Contractions are small, rapid, and of moderately high tonus.
C. After 80 I.U. of progesterone. Contractions of low tonus, slow, and great amplitude. Fourth day of flow. Secretory endometrium.

between them is lengthened. Uterine tonus is reduced, and the response to pituitrin is more marked. During this phase the uterus never fails to respond to pituitrin. Often superimposed on these large slow contractions are smaller, shorter waves that resemble the follicular-phase contractions. The contractions continue to increase in amplitude and reach their maximum height on the first or second day of the menstrual flow (fig. 1*B*). They then gradually decrease in size and duration until about the end of the first week, when they are replaced by the small rapid follicular-phase contractions (fig. 1*C*). The above changes may be summarized as follows.

Phase	Amplitude	Interval	Tonus	Reaction to Pituitrin
Follicular	Small	Short	High	±
Luteal	Large	Long	Low	+ +

Correlation of these contractility changes with the endometrial cycle indicates the existence of a definite parallelism. The endometrium, however, reflects the ovarian changes much sooner than the myometrium. The latter lags about a week behind the endometrium, and continues to display luteal-

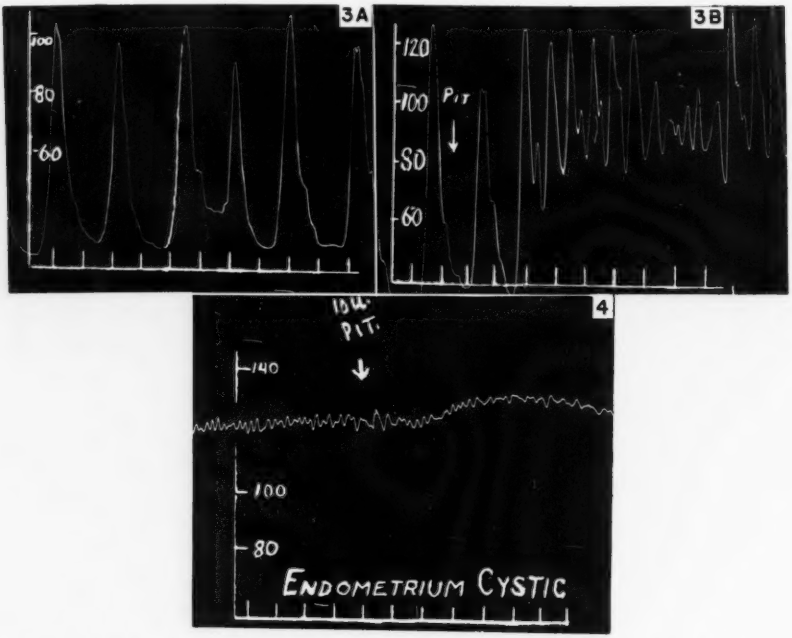

Fig. 3*A*. Patient *R.S.* Oligonmenorrhea. Contractions during first day of flow. Typical luteal phase type; low tonus, great amplitude, slow.
B. Oligomenorrhea. First day of flow. Reaction to 10 u pituitrin intramuscularly.
Fig. 4. Patient *R.P.* Cystic glandular hyperplasia of the endometrium of many years' standing. Typical follicular-phase contractions.

phase characteristics for some time after the secretory endometrium has been shed and replaced by a new proliferative endometrium.

Amenorrhea

Three cases of long-standing amenorrhea were studied. In each there was either a persistent absence of contractility, or at best an occasional minute wave. The tonus was uniformly high and the reaction to pituitrin negative or minimal. The following case merits detailed description because it served as a carefully controlled experiment in which the two types of contractions were artificially produced.

Case 1. M. M., aged 37 years, white, married, never pregnant, had attended the Vanderbilt Clinic since 1934 for primary amenorrhea. The breasts and external

genitalia were slightly hypoplastic, the uterus was anteverted, freely movable, and normal in length by sound (2¾ inches). The adnexae were not palpable. There was no obesity or hirsutism. The B.M.R. ranged from —3 to —23 per cent. Several hormone determinations revealed a persistent absence of follicular and follicle-stimulating hormones in the urine. A number of endometrial biopsies always revealed an atrophic endometrium. These findings indicate a very marked degree of ovarian and pituitary gonadotropic hypofunction so that, for practical purposes, the patient may be considered as a castrate.

Uterine contractility studies made at weekly intervals for a period of 8 weeks persistently showed a complete absence of contractions. Pituitrin evoked no response and the tonus of the uterus was always high (fig. 2*A*). Estradiol benzoate (50,000 I.U. twice weekly) was then administered for a period of 8 weeks. At the end of the first week minute contractions appeared. By the fourth week the contractions were fairly typical of the follicular-phase (small and rapid); the response to pituitrin slight, at times negative (fig. 2*B*). An endometrial biopsy revealed a proliferative endometrium. Then progesterone (10 I.U. twice weekly) together with 50,000 I.U. of estradiol benzoate was administered for a period of 4 weeks. Two days after the first injection the patient began to bleed moderately for 3 days. The contractions grew progressively larger and longer, the response to pituitrin was considerably increased and there was a noticable drop in tonus. Three weeks after the administration of progesterone was started, bleeding accompanied by mild abdominal cramps occurred. Figure 2*C* demonstrates the large contractions recorded on the 4th day of this flow. A biopsy revealed a typical secretary endometrium. The administration of progesterone was stopped but the estradiol benzoate was continued; the contractions and the endometrium returned to the type characteristic of the follicular-phase.

Oligomenorrhea

When the periods occur at infrequent intervals, the delay, as was shown in a previous study (3), is limited to the follicular phase; the length of the luteal phase rarely exceeds 2 weeks. In keeping with these facts, the contractions remained persistently small for weeks and months until finally, about 10 days before the beginning of the flow, large slow waves made their appearance and continued throughout the luteal phase. The latter contractions, particularly on the first day of menstruation, often reached tremendous amplitudes (figure 3 *A* and *B*).

Ovulatory Bleeding

In cases of hypermenorrhea associated with ovulatory cycles (bleeding from a secretory endometrium), no significant deviations from normal uterine contractility were noted. This is in agreement with an earlier opinion (4) that the cause of functional bleeding is independent of the type of function the uterus or the ovaries, in that bleeding may occur from any type of endometrium.

Anovulatory Bleeding

In a case of anovulatory bleeding of many years' duration, associated with a cystic endometrium, contractility studies at weekly intervals over a period of 14 weeks persistently revealed the characteristic small follicular-phase con-

tractions (fig. 4). The uterine tonus was always high and the response, if any, to pituitrin was slight.

The weekly administration of 10 to 20 I.U. of progesterone had no appreciable effect on uterine contractility, although bleeding and shedding of the cystic endometrium occurred promptly and was followed by mild secretory activity in the endometrial glands. Perhaps larger doses of progesterone are necessary to produce a myometrial response.

Dysmenorrhea

Examination of 72 tracings from 14 patients with functional dysmenorrhea revealed no appreciable differences in the contractions as compared to those in women with painless menstrual cycles. It is interesting that in our entire experience with dysmenorrhea we have always noted its association with an ovulatory cycle as shown by the presence of a secretory endometrium. This has been so consistent that we now believe the presence of functional dysmenorrhea indicates the occurrence of ovulation. Furthermore, in a large experience with anovulatory bleeding we have never observed dysmenorrhea, although the bleeding was often very profuse and accompanied by the passage of large clots. It is also worthy of note that dysmenorrhea occurs at the time when the uterine contractions achieve their greatest amplitude, i.e., just preceding and during the first few days of the menstrual flow.

In 1934 we observed that the administration of large doses of estradiol benzoate (50,000 I.U. on the 5th and 12th days of the cycle) gives satisfactory or complete relief of dysmenorrhea in about 70 per cent of the cases (5). More recently we have obtained similar results by the use of testosterone propionate (25 mg. per dose). We are at present engaged in studying the effect of these injected hormones on uterine contractility in order to determine if the relief of menstrual pain is attributable to changes in the character of the contractions.

Our experience with progesterone therapy in dysmenorrhea has been very disappointing. This is to be expected with our present understanding of the effect of progesterone on the myometrium.

DISCUSSION

When the ovaries cease functioning, the myometrium becomes inactive and undergoes atrophy. The small rapid follicular-phase contractions perhaps represent the normal spontaneous activity of the uterus. Primed by estrogenic hormone, the myometrium is at all times ready to respond to progesterone. It is interesting to speculate as to what purpose the large luteal-phase contractions might serve. Several possibilities have occurred to us but one that appears reasonable is that these waves are perhaps the precursors of the painless Braxton-Hicks contractions responsible for the development of the lower uterine segment during pregnancy. That these contractions are present from the beginning of pregnancy is suggested by the occurrence of Hegar's sign as early as the 6th week. Thus far, we have had no opportunity to study uterine contractility during the course of pregnancy, so that we are unable at this time to present proof of our hypothesis.

Another interpretation also suggests itself. It has usually been assumed

without any experimental proof that the waves of contraction in the human uterus begin somewhere in the tubes and travel downwards toward the cervix. The progress of the spermatozoa up the uterus and toward the ovum would therefore be opposed by two forces: the downward ciliated sweep toward the internal os, and the contractions that begin in the tubes and travel towards the cervix. If we add to these the probability that the duration of the fertilizing power of sperm is relatively short—much less than 48 hours—and that the energy supply within the sperm is also limited, we are forced to the conclusion that some mechanism probably exists which favors the upward progress of spermatozoa. We therefore suggest that during the follicular phase of the cycle the contractions may pass from the cervix toward the tubes. It is conceivable that the cervical ganglion acts as the pacemaker. Blotevogel (6) has described definite changes in this ganglion in response to estrogenic hormone.

The problem during the luteal phase is decidedly different. The problem now concerns the transport of the fertilized ovum through the tube and into the uterine cavity. The progress through the tube, during which the early stages of embryological development take place, is slow. Such transport would be favored by slow contraction waves spreading from the outer end of the tube towards the cervix. It is suggested that progesterone causes a reversal in the direction of the uterine contractions. That progesterone is capable of producing a marked alteration in uterine contractility was recently shown by Kennard (7). This author observed that stimulation of the hypogastric nerves of the non-pregnant cat normally resulted in relaxation of the uterus. The same effect was obtained by injecting adrenalin. When progesterone was administered, the identical stimuli had the opposite result, i.e., the uterus was stimulated to contract. In pregnancy, the uterine response was the same as that obtained in the non-pregnant animal injected with progesterone.

It was noted in these studies that the contractility changes lag behind the histological changes in the endometrium. Following ovulation, histological changes denoting the luteal phase can be detected in about 48 hours (biopsy curette). At this time the myometrium still presents contractions characteristic of the follicular phase. Typical luteal-phase contractions appear several days later. A similar myometrial lag is noted in the change from luteal to follicular-type contractions several days after the termination of bleeding. This suggests that the endometrium is more sensitive to hormonal changes than the myometrium. We (8) have previously observed that in cases of precocious sexual maturity the uterus retains its infantile size and proportions, even though the patient is menstruating regularly. Little is known of the mechanism of the action of estradiol and progesterone upon the uterine musculature. Genell (9) points out that the following possibilities have been investigated to some extent: *1)* A direct hormonal effect upon the uterine musculature. *2)* By way of the autonomic nervous system (10). *3)* By way of changes in the ionic concentration of the muscle and the blood. *4)* By way of metabolic changes in the muscle. *5)* Through vascular changes in the myometrium. *6)* By way of the posterior pituitary gland. Decisive data are not as yet available.

If a functional myometrial cycle is demonstrable in the normal woman, then we should be able to duplicate it in the castrate. The case of primary amenorrhea fulfilled this requirement. We were able to duplicate the normal

myometrial cycle by the administration of estradiol benzoate and progesterone in proper amount and sequence. When this patient first came to the clinic, we produced cyclical bleeding at regular intervals by the continuous administration of estradiol benzoate. The periods were never painful, and the endometrium was always of the proliferative type. When progesterone was given in order to bring out the luteal phase contractions, the menstrual period was very painful.

Our experience with progesterone in cases of habitual and threatened abortion has been rather disappointing. This type of therapy was originally suggested because of the supposed inhibitory effect of progesterone on the myometrium. In the light of our present knowledge, its use appears illogical except in so far as it might favor nidation and placentation.

SUMMARY

The contractions of the non-pregnant human uterus were studied, in vivo, by means of the intrauterine bag; 29 patients were studied and 187 tracings were obtained. A functional myometrial cycle was demonstrated. The follicular phase of the cycle is characterized by a high tonus and small rapid contractions. The luteal phase is characterized by a low tonus and by slow contractions of great amplitude. These characteristic contractions are preceded for several days by the endometrial changes characteristic of the particular phase of the cycle (the myometrial lag). The characteristic myometrial cycle was produced in a case of primary amenorrhea by the administration of estradiol benzoate, followed by progesterone.

Patients with anovulatory cycles, associated with a proliferative or cystic endometrium, show only follicular-phase contractions. These pathological states are characterized by an absence of corpus-luteum hormone.

Patients with dysmenorrhea present the same type of contractions during the various phases of the cycle, as do women with normal painless periods. No significant variations in either tonus or rate or amplitude of contractions were demonstrable in dysmenorrhea. Only patients with corpus-luteum function can have (functional) dysmenorrhea.

The uterus reacts to pituitrin during all phases of the cycle. The most intense reactions are obtained during the luteal phase.

We are indebted to Professor Benjamin P. Watson for his constant encouragement and advice, and for placing the large material of the Sloane Hospital for Women and the Vanderbilt Clinic at our disposal.

We also wish to thank Miss Belle Francis for her tact and skill in the handling of these patients.

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STUDIES ON THE PITUITARY WEIGHT OF RATS INOCULATED WITH A TRANSMISSIBLE TUMOR

J. M. TWORT AND M. LASNITZKI
From the Cancer Research Department, University of Manchester
MANCHESTER, ENGLAND

These investigations were undertaken firstly, to find out whether there was any difference in the weight of the pituitary gland in those rats relatively susceptible to inoculation with a transmissible tumor, as compared with those which were not susceptible. Wyeth (1) found that in human cancer cases the weight of the pituitary in both sexes and all age classes was increased. Our second object was to try and confirm the results of several authors (2, 3, 4, 5) regarding pituitary variations pertaining to sex.

METHODS AND RESULTS

The rats, numbering 163, were inoculated subcutaneously with the Jensen rat sarcoma (injection of minced material), and the animals killed in batches from 13 to 24 days after inoculation. Weight of tumor and total body weight (without tumor) were determined, the pituitary gland being subsequently removed. A portion of the base of the skull with the pituitary in situ was placed in absolute alcohol for 24 hours. The gland was then dissected from the base and kept for 6 days in a test-tube placed in a dry cupboard, and then weighed on a microbalance. The brain and spleen were also removed, and weighed immediately on a torsion balance.

In the first place, the rats which had developed tumors, and those which had not, were divided into 4 sub-groups according to body weight. The mean of the ratio of pituitary weight to body weight was then determined for each sub-group, but no very significant difference between tumor-bearing and tumorless animals was apparent by this procedure.

As the presence of the tumor sometimes profoundly affects the general health of the animal and thereby the body weight, it was felt that the ratio of pituitary weight to brain weight might reveal a more significant difference between the 2 groups of animals. The tumor-bearing and tumorless groups of rats were therefore divided into 5 sub-groups, those animals with brain weights of 1.40 to 1.50 gm. comprising *group 1,* those of 1.50 to 1.60 *group 2* and so on, all of *group 5* having a brain weight above 1.80 gm. The mean of the ratio of pituitary weight to brain weight was then determined for each sub-group. The values obtained in this way are illustrated in figure 1, each of the 10 sub-groups being further subdivided into males and females. Nine pairs of sub-groups only are available for comparison, as no tumorless male animals were present in *group 1.* Altogether 88 tumor-bearing animals (41

males, 47 females) were compared with 66 tumorless animals (24 males, 42 females).

It will be seen that in 8 of the 9 pairs the pituitary weight is relatively less among the tumor-bearing than among the tumorless animals, our results being uniform on the whole, in spite of a considerable difference in the number of animals under comparison. Quantitatively, the decrease varied between 9 and 37 per cent. However, in *group 3* (females), there was an increase of 10 per cent. If *group 3* is included, the average deviation for all the female groups was a decrease of about 17 per cent, whereas for the male groups it was about 18 per cent. On the other hand, if we mass all the individual animals

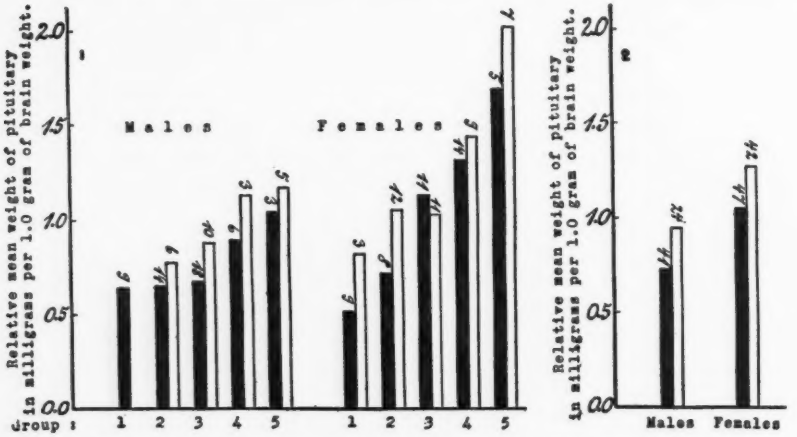

Fig. 1. Weight of rat's pituitary in relation to brain weight, by groups. Solid black bars—tumor-bearing rats; open bars—tumorless rats. Figures at the top of each bar represent number of animals.
Fig. 2. Same as figure 1, but with animals massed.

of each sex, we find an average decrease of about 23 per cent among the males, and again of about 17 per cent among the females. These deviations are illustrated in figure 2.

Both figures also show clearly that there is a sex difference in the relative weight of the pituitary gland, that of the female being markedly greater than that of the male. The values illustrated in figure 2 show that, on an average, the female pituitary is by about 45 per cent larger than that of the male among the tumor-bearing animals, and about 34 per cent among the tumorless. This will also be seen in every relevant case illustrated in figure 1, with the exception of *group 1* (tumor bearers), the increase of the female gland as a rule varying between 20 and 70 per cent. Furthermore, it will be seen that, in both sexes, the relative weight of the pituitary increases as brain weight increases (i.e., as age of animals increases), but that this increase is more pronounced among the females. Also, our findings in connection with sex are somewhat similar if body weight is substituted for brain-weight. In this case, however, the relative weight of the male pituitary shows a tendency to decrease as the body weight increases. Generally speaking, although our results agree with the findings of other investigators working with other species of animals,

there are certain differences. Thus investigations with mice have disclosed the fact that the female pituitary, although appreciably larger than that of the male relative to body weight, did not usually appear to be larger relative to brain weight (5).

That the pituitary gland of tumor-bearing rats is relatively smaller than that of the tumorless may be due to an influence of the pituitary, directly or indirectly, on the susceptibility of the rat to tumor development, animals with smaller glands being the more susceptible. Or the development of the tumor itself may lead to a diminution in the size of the pituitary, again either directly or indirectly. Meanwhile, the best explanation appears to be that the growing tumor indirectly leads to a relative loss in pituitary weight. Such an assumption is indicated by the connection it has to another group of facts. Edwards and Wright (6) have recently reported that extirpation of the spleen in (female) rats caused an increase in the size of the pituitary. On an average, the relative weight of the organ (in regard to body weight) was increased by 27 per cent. On the other hand, it is known that the development of a transmissible tumor in rats and mice causes an increase in size of the spleen. In our animals, enlargement was also rather pronounced, the relative weight of the spleen (in relation to body weight) being, on an average, about 45 per cent greater among the tumor-bearing animals than among the tumorless, with no clear distinction between the two sexes. If, therefore, a loss of splenic tissue causes an increase in the relative weight of the pituitary, it would be expected that an increase would have the opposite effect. Thus we conclude that a growing tumor causes primarily hypertrophy of the spleen, which in turn leads to diminution of the pituitary gland.

In order to ascertain a possible relation between the size of the tumor and the relative pituitary weight, the tumor-bearing animals, both males and females, were divided into two groups, those with tumors below and above 10 gm. in weight. In the former group the average tumor weight in grams was: males 5.3, females 6.2, whereas in the latter group it was 18.0 and 18.5 respectively. From our analyses there was no definite indication of a relation between size of tumor and reduction of pituitary weight. It therefore appears that the process leading to a diminution of the pituitary gland takes place chiefly during the early stages of tumor development.

Lastly, it is to be noted that our result is contrary to the findings of Wyeth (1), obtained with the pituitary in human cases of cancer. Again, the inference is that the nature of spontaneous tumors is presumably somewhat different from that of transmissible tumors.

SUMMARY

A number of rats previously inoculated with Jensen rat sarcoma were divided into 2 groups according to receptivity. Each group was further divided into sub-groups according to brain weight, the sub-groups being in turn separated into males and females. It was found that, on an average, the ratio of pituitary dry weight to brain weight was usually smaller in the groups of animals bearing tumors than in those without. When all the animals of each sex were massed, the average decrease was about 23 per cent among the males,

and about 17 per cent among the females. It is suggested that a connection exists between the diminution of the pituitary gland and the increase in the size of the spleen observed in tumor-bearing animals.

In every group except one, the relative weight of the female pituitary was found to be greater than that of the male organ. On an average, the increase was about 45 per cent among the tumor-bearing animals, and about 34 per cent among the tumorless animals.

The authors wish to acknowledge their indebtedness to Dr. A. Lasnitzki for his valuable suggestions in connection with this paper.

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CLINICAL, HORMONAL AND HISTOLOGICAL CONDITIONS IN A CASE OF HERMAPHRODITISM (FEM. EXT.)

J. CHARVÁT, E. KODÍČEK AND O. SCHUBERT
From the Second Medical Clinic, Charles University, and the State Institute of Hygiene
PRAGUE, CZECHOSLOVAKIA

Since the first case of virilism was described (1756), a number of authors have occupied themselves with this syndrome. A tumor or hyperplasia of the adrenal cortex was being established in cases of inter-renalism. In recent times may be mentioned the work of Walters, Wilder, Kepler (1), and Crooke (2). It was Cushing (3, 4) who drew attention to the hypophysis by describing a number of cases of virilization with a finding of basophilic adenomas in the hypophysis. Virilization has been observed also in cases of tumors of the ovaries of the type of Meyer's arrhenoblastoma (5).

In this paper is described a case of virilism in a woman whose state was investigated by clinical and laboratory methods and verified by postmortem examination. Having followed the secretion of sexual hormones during normal and pathologic states by means of biological assays, we selected this case which, owing to its great rarity, cannot be placed in any of the categories investigated by us. Also in this case we carried on the investigation of the secretion of gonadotropic, estrogenic and male hormones for a space of one month in a way similar to that done by Siebke (6), Frank (9), and recently by Damm (8), Gustavson and Green (9).

CASE REPORT

J.Z., a woman aged 22 years, was admitted for examination on Oct. 4, 1933. She had never been ill, and up to about 6 years of age developed like other children. At that age, a period of rapid growth set in, so that she was the tallest in her class. Except for being emaciated, she experienced no discomfort. At 15 years of age, her girlish form began to alter, she grew stronger, broad-shouldered and muscular. The mammae failed to develop; there was only a suggestion of these. At the same time, hair began to appear on the face (she was obliged to shave once a day), as well as on the chin, upper lip, the arms, legs and trunk. Simultaneously, the middle lobe of the thyroid gland grew larger, the voice became coarser, and these changes continued to develop. At the age of 17, she had attacks of nausea and vomiting for a whole year, but otherwise felt healthy. Menstruation did not appear, not even a trace of vicarious bleeding. Subsequently, the patient complained of restlessness and her hands and legs shook a great deal.

Physical examination. The patient was of medium height, 166 cm.; the span of the upper extremities measured 160 cm., the lower extremities 75 cm. in length. On the whole, she looked well-nourished; fat was distributed all over the trunk and face, and in a smaller quantity over the extremities. The mammae were small and lean.

They lacked glandular tissue, were masculine in character, and the nipples were rather large, little pigmented, and not erectile. The general impression was that of a man. There was considerable hirsuteness on the forearms, as well as on the face, nose, cheeks and chin, and the growth of hair on the mons veneris reached up to the naval. Scalp hair was very fine, and kept receding; the forehead became higher, and the face seemed that of a young man. The eyebrows were conspicuously

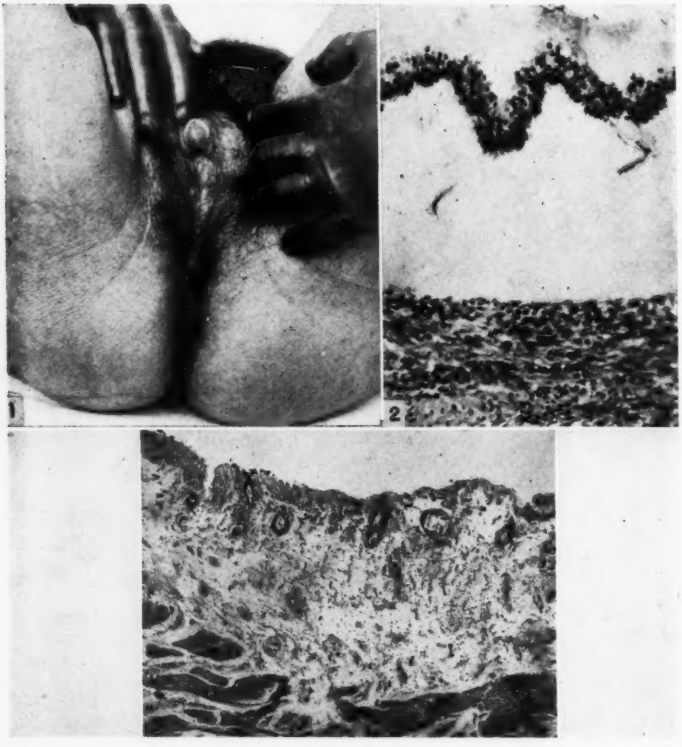

Fig. 1. External genitals of patient *J.Z.*
Fig. 2. Peeling and degeneration of the granulosa in the follicular cyst of the ovary. There are no signs whatsoever of luteinization in the wall of the follicle. x145.
Fig. 3. Endometrium strongly hypoplastic, almost devoid of glands. x30.

thick, the voice coarse and deep. Psychically, the patient appeared normal, but rather depressed. She felt herself to be a woman.

The middle lobe of the thyroid gland, enlarged to the size of a nut, was tough and did not pulsate. There were red-purple striae on the sides of the abdomen. Blood pressure was 165/100 mm. Hg, the pulse rate 96/min., the weight 69.50 kg. Red blood count was 5,480.000, white blood count 8.600, hemoglobin index 1.05. Roentgenograms of the sella turcica, of the renal region (i.v. pyelography), and of the heart and lungs were normal. There was no osteoporosis in the bones.

Laboratory studies. The urine was normal, the Wassermann reaction negative. The amount of creatinine in the serum was 1.4 mg. per cent, calcium in serum 8.3 mg. per cent, cholesterol 177 mg. per cent, residual nitrogen 30 mg. per cent, and the alkaline reserve (van Slyke) 53.8.

Strauss dilution and concentration test results were normal. The blood sugar values after ingestion of 50 gm. of glucose were as follows.

	30 min.	*60 min.*	*120 min.*	*180 min.*
Glycemia 0. 86%	1.44%	2.25%	1.2%	0.9%
Glycosuria 0	0.2%	2.0%	0	0

The glycemic curve after subcutaneous injection of 1 mg. of adrenalin was normal. The blood pressure did not change. There was no glycosuria.

Specific dynamic effect was tested by feeding 200 gm. of ham. The B.M.R. at the beginning was +37 per cent; after 1 hour it was +39 per cent, after 2 hours +50 per cent, and after 3 hours +48 per cent. The metabolic rate before a work test was 37 per cent. Two minutes after doing work it was +75 per cent, after 30 min. +55 per cent, after 60 min. +45 per cent, after 120 min. +44 per cent. These findings are essentially normal. The metabolic rate before a subcutaneous dose of 0.3 mg. of pilocarpine was +35 per cent; after 30 min. it was 52 per cent, after 60 min. +43 per cent, after 90 min. +37 per cent, and after 120 min. +45 per cent.

The ophthalmological findings were normal. A gynecological investigation showed a penis-shaped clitoris hidden by a foreskin under which there was a soft slit about 0.5 cm. in length, resembling the outer orifice of the urethra (fig. 1). This was atretic to sounding. In place of the vagina there was a mere slit, about 3 cm. in depth, and on its front side the urethral orifice. The labia majora were developed normally; on palpation nothing could be felt in them. By rectal examination, in the small pelvis, somewhat to the right of the locality where the uterus is normally found, a sensitive round corpuscle was situated in the proximodistal direction with a slight deflection to the right. Delicate fibres probably extended from its proximal end.

Hormonal findings in the urine. From April 16 to May 14, 1934, the urine was examined nearly every day for its content of sexual hormones (fig. 3). These tests were made in two ways: for higher values (more than 80 R.U. per liter), by the original concentration method of Zondek. This method, however, meant considerable loss. Our results obtained from direct injection of urine into rats are thus more reliable, and we are able to say that our patient had not more than 80 R.U. per liter of urine. With concentrated urine the assays showed less than 5 R.U. per liter, but we hesitate to say that there was actually less than this amount, in view of the considerable loss of hormone by this method. On the whole, it is safe to say that instead of the gonadotropic hormones being increased they were in all probability rather lowered. Accordingly, the patient secreted estrogenic hormone in a certain curve, its amount, judging from our experience, being almost pathologically increased on April 25-26. We shall recall these findings later on, in the discussion.

The male hormone was titrated according to the method of Koch and Womack (10). We found a considerable increase of male hormones in the urine as compared with our findings in normal women, this fact being in agreement with Bühler (11), who also found a higher amount of male hormones in women affected by hirsutism.

After the patient's discharge from the clinic, the region of the hypophysis was treated by x-rays in two series of 6 sessions each, after which the region of the adrenals was also irradiated, but without any noticeable effect. Since the patient's state showed no improvement, laparotomy was performed by Dr. Jirásek on July 14, when the right adrenal gland, obviously enlarged, was removed. As a matter of fact, we assumed hyperplasia or an adenoma of the adrenal cortex. The

patient fell into a severe coma of cortical insufficiency 24 hours after the operation, and could not be saved even by large doses of adrenal hormones.

Postmortem findings. Through the courtesy of Dr. Šikl, of the Pathological-Anatomical Institute of the Charles University, we have received the following findings: Pseudo-hermaphroditismus externus femininus; hirsutism; hypoplasia of inner genital organs; extirpation of the right adrenal gland and denudation of the left; postoperative hemorrhagic cyst in the left adrenal gland; turbid swelling of organs; activation of the pulp of the spleen (postoperative); a slight edema of the brain, hyperplasia of the thymus, cystic adenoma of the thyroid gland, and lipoid necrosis of pancreatic tissue. As for the macroscopic findings, the skull showed no pathologic alterations, the weight of the hypophysis was somewhat enlarged (1.05 gm.), and the thyroid gland was noticeably enlarged by a nut-sized nuclear body, weighing 75 gm., in the middle lobe. The adrenals were found as mentioned above. The ovaries were in normal position, the right one slightly smaller, 2.5 x 2.0 x 1.0 cm. They had a smooth albuginea, with several perceptible scars. The tubes were normally developed, the uterus very small, only 4 cm. in length, infantile, the lumen filled with mucous substance, particularly in the cervical part. The vagina measured only 6 cm. in length, its maximum circumference was 5.5 cm., and in the distal part only 4 cm. The urethra began close over the introitus of the vagina and was encircled by a layer of tissue 0.5 cm. thick, roughly resembling the prostate gland. No mascroscopic traces of aberrant testicular tissue could be detected. The urinary bladder showed no alterations.

Histological examination of the right adrenal gland disclosed a normal condition. All the parts examined contained only cortex; no trace of medullary tissue was found. The structure showed some alterations which were in all probability due to the operation. The only material finding was a small nodule, which microscopically corresponded to a ripe cortical adenoma. Assuming that we obtained the entire tissue after the operation, the weight of the right adrenal gland thus totalled 14.7 gm. As for the left adrenal gland, the central hematoma had entirely disintegrated the medulla. The cortex showed regressive alterations of various intensities, so that it was very difficult to reconstruct the original arrangement and volume. There seemed to be a comparatively small amount of pigment in the cells of the reticular zone. A nodular hyperplasia of the size of a pea occurred in one block. The microscopic structure of the hyperplastic tissue corresponded partly to the fascicular, and partly to the reticular zone, and did not differ in any way from current findings of this kind.

Hypophysis. The arrangement of the adenohypophysis was regular, and adenomatous proliferation occurred nowhere. Among the cells there was a marked increase of basophilic elements, the eosinophilic cells being few in number. In the intermediary part, there were comparatively voluminous cysts, but on the whole this finding was within the limits of the normal.

Ovary. Primordial follicles were present in the usual number, and isolated follicles at different stages of maturity could be seen. On the other hand, there was a comparatively large number of follicles resembling ripe follicles or somewhat larger, which were filled with a liquid of homogeneous or finely granular nature. Some of these had a well-developed granulosa, but the ova were either in a state of degeneration or completely lacking; also the granulosa showed different stages of degeneration with dissociation of the cells and their transformation into homogeneous eosinophilic globules (fig. 2). In some cases, the granulosa had disappeared entirely, and the theca was in a state of mild proliferation; such follicles were generally beginning to shrivel. Lastly, foci could be found consisting entirely

of proliferated theca cells, thinly arranged, but without a central cavity, these being clearly the remainders of atretic follicles. Any trace of luteinization was absent, nor did bleeding into the follicles occur anywhere (corpus hemorrhagicum). Consequently, no corpora lutea could be found, nor were there present anywhere the typical whitish corpuscles, except for one rudimentary hyaline corpuscle of doubtful significance.

On the whole, the histological findings in the thymus, spleen and liver were normal. The degree of involution of the thymus corresponded, at its highest, to the pubertal stage.

DISCUSSION

By clinical examination, we thus found hirsutism with hermaphroditism, a typical gynecological condition, colloid goiter with hyperthyroidism, hypertension (165/100 mm. Hg), a lowered carbohydrate-tolerance, increased basal metabolism, and a lowered oxygen consumption after 0.3 mg. of pilocarpin. The rest of the findings were normal. We assumed hyperplasia of the adrenal cortex. The operation and, later on, the autopsy showed a slightly enlarged left adrenal gland, but the weight of the right adrenal hardly exceeded the normal variation range. Conclusions were difficult owing to the postoperative changes. The finding on the thyroid was in agreement with the clinical findings. The postmortem findings on the internal genitals completed the clinical examination. We found a great hypoplasia of the uterus. The abdomen contained no aberrant tissue, either cortical or testicular, such as described by Kolodny (12). The histologic findings were of great interest: in both adrenal glands small cortical adenomas were found, which however might well be found also in normal persons. The thymus was hyperplastic, with a slight involution. The hypophysis was somewhat heavier, with basophilic cells occurring in higher proportion than normal, but no adenoma was found.

Particularly important were the results of the histological examination of the ovaries and of the hormonal analysis in the urine, the two completing each other. The titrations for estrogenic hormone, carried on for the period of a whole month, have always yielded rather high figures not in agreement with other findings given by authors in cases of primary amenorrhoea. On the contrary, they are even higher than we have found in normally menstruating women. These findings might otherwise justify the hormonal diagnosis of metropathia polyglandularis hyperplastica, but in our case the histological findings in the uterus as well as those in the ovary completely exclude this diagnosis. Thus, on the whole, there are ripening follicles that fail to attain maturity and consequently perish atretically, but corpora hemorrhagica, or even corpora lutea, are totally absent. Such alterations are assumed by Siebke (13) in diseases marked by delayed menstruation. He speaks of a growth of follicles perishing atretically without maturing. He found in such cases a transient, repeated increase of the estrogenic hormone in the urine above the normal amounts, and followed by a great and sudden decline to subnormal limits (caesures). An apparently analogous state has been described by Frank (7, 14) in the titration of hormones in the blood as a sub-threshold-cycle.

Our case, however, was different in nature. We found a primary amenorrhea and virilism associated with higher amounts of estrogenic hormone

in the urine, for which no other explanation existed but that several follicles ripened simultaneously, and secreted a higher amount of estrogenic hormones. Since these follicles perished atretically, however, the hormonal cycle was incomplete. Our assumption was verified by the histological findings in the ovaries, which was, as far as we know, not the case in Siebke's paper. But the difference between our curve and that of Siebke was that we had no declines (caesures) between the rises in hormone amounts such as he described in his cases with a prolonged cycle.

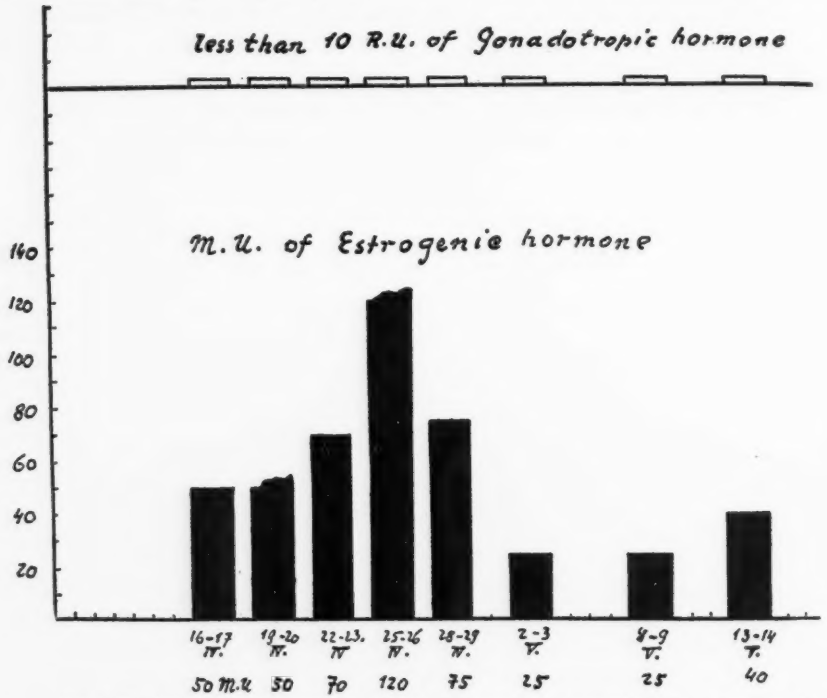

Fig. 4. Elimination of gonadotropic and estrogenic hormones. In the case of estrogenic hormones, the graph of their secretion proved very interesting. It rose from 50 M.U. to more than 120 M.U. (on April 25-26), was then lowered to 25 M.U., and towards the end rose again to 40 M.U.

This phenomenon might be due to the lack, or even absence of, gonadotropic hormone, in particular of its luteinization factor, an hypothesis that would agree with our titration results of gonadotropic hormone in the urine (fig. 4). Even the adrenal cortex might have something to do with this phenomenon. Migliavacca (15) found considerable luteinization in the ovaries after injecting adrenal cortex extracts. A similar influence has been established by Winter, Reiss, Balint (16, 17, 18), and Hohlweg (19). According to the last papers of Hoffmann (20), marked luteinization in the ovaries can be found in rats after injection of adrenal extracts, together with a small dose of gonadotropic hormone, only folliculinization being produced with the same small dose of gonadotropic hormone if the adrenal extract is not added. The

papers of these authors show the great influence of the adrenal cortex upon the normal correlation of sexual hormones. In our case we might assume that the function of the adrenal cortex suffered impairment.

The amount of male hormone we found in the urine exceeded that usually found in normal women. As we shall discuss this question in detail in another series of our investigations, we simply state here the results obtained. In cases of hirsutism, we have almost invariably found a higher amount of male hormone in the urine, whether castrate seminal vesicles or capon's comb have been used as criteria. In many cases the amount of male hormone in the urine was greater than even that in healthy males. The fact that Reichstein (21) succeeded in isolation from the adrenal cortex adrenosterone, a cristallic steroid which possesses the effects of male hormone, seems to us to be very significant for our findings.

The postmortem findings on the internal genitals absolutely contradicted the histologic examination on the ovaries and our titration results. In view of the considerable amount of estrogenic hormone secreted, we should have expected if not hyperplasia at least proliferation in the uterus (22). But the pathologist found a hypoplastic uterus with an entirely inactive mucous membrane (fig. 3).

Thus we see cases in which even a sufficient amount of estrogenic hormone does not influence the growth of the uterus, and so feel entitled to infer that, in addition to the estrogenic hormone, there exist other factors (which may also be endocrine in nature) influencing the growth of the uterus, and other changes in the internal genitals. On the other hand, it must be assumed that, in some cases, the internal genital organs are unable to respond to estrogenic hormone. This affection may be either constitutional or acquired (23).

An interpretation of the etiology of hirsutism in this case is very difficult. Authors do not agree as to whether the changes in the adrenal glands should be considered responsible for hermaphroditism, or as secondary changes due to the same cause as hermaphroditism. In some cases no changes at all have been found in the adrenals (24). Our patient's adrenals were somewhat enlarged, and small cortical adenomas were found in them, but in our opinion this circumstance does not satisfactorily explain the etiology of this case of hermaphroditism. The hypophysis did not show any noticeable changes either, and no adenomas were found, even in serial examinations. The hypophysis was only heavier, and the basophilic cells were increased diffusedly, which, however, may be a secondary phenomenon (24, 25, 26).

SUMMARY

We have described a case of pseudo-hermaphroditism (fem. ext.) associated with hirsutism, which has been examined clinically, histologically and by serial titration of sexual hormones (gonadotropic, estrogenic and male hormones) in the urine during a period of one month. The amount of gonadotropic hormone was small, and estrogenic hormone was secreted in an increased quantity; an increased proportion of male hormone was found in the urine. At the same time, however, the ovary was small with numerous immature follicles, and without the slightest trace of luteinization; the uterus

was small and hypoplastic with an atropic mucous membrane. The adrenals were slightly enlarged with cortical adenomas, and basophilic cells in the hypophysis proliferated. The histologic finding in the ovary is in accordance with the titration results of sexual hormones. The condition of the uterus, however, showed a paradoxical phenomenon: in spite of the increased proportion of estrogenic hormone it was in a state of complete inactivity. We believe that the state of the ovary (no luteinization, etc.) was due either to lack of gonadotropic hormone, or to the impaired function of the adrenal cortex. For reasons unknown, the uterus either did not respond to estrogenic hormone, or some other factor (endocrine) was lacking which may be necessary, apart from estrogenic hormone, for the uterus to develop normally. A considerable amount of male hormone was found in the urine, as has been true in other cases of hirsutism, occasionally being even higher than in normal values. The relation to the adrenal cortex is pointed out.

We are deeply indebted to Dr. Šikl, head of the Pathological Institute in Prague, for his assistance in the histological examination of the case.

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CLINICAL AND LABORATORY NOTES

A TECHNIC FOR HYPOPHYSECTOMY OF THE MOUSE*

FRÉDÉRIC THOMAS†

Hypophysectomy in the rat has become part of the routine of some laboratories, but this operation in the mouse, judging from the scanty literature, seems to have been considered as being relatively impracticable. The only mention we could find of its performance is in two short papers by Selye, Collip and Thomson ([1, 2]). These authors report the results of experiments performed on hypophysectomized mice, but content themselves with mentioning that the operative technic was the same as their technic for the rat, in which a hole is bored through the base of the skull with a dental burr.

Any technic based on this procedure and applied to the mouse encountered two almost insurmountable obstacles, in our experience: (a) the great immediate and postoperative mortality occasioned by incidental injuries associated with the boring of the skull-base, and (b) the impossibility of completely and thoroughly sucking out the flat, oblong pituitary of the mouse through a round hole much smaller than the gland itself. Before beginning with the extirpation, therefore, it seemed necessary to find a method which would prevent fatal cranial injury, and at the same time expose the whole pituitary in its anatomical site.

Choice of animals, anesthesia and optical equipment. It is technically possible to remove the pituitary from mice of every age, but we have found that extirpation was easiest by far in mice weighing between 10 and 20 gm. A short (20 min.) but effective anesthesia was obtained by the intraperitoneal injection of 2.5 per cent solution of Avertin in saline. The dosage was 0.9 cc. per 100 gm. body weight. The optical equipment employed has become classical for hypophysectomy in the rat. The magnification 12x was used throughout the operation.

Instruments. Few instruments were used: 1 pr. small straight scissors; 2 small straight forceps; 2 blunt curved hooks for retraction and blunt dissection; 2 minute hooks used to break or separate the bone; and 2 curved glass suction tubes of unequal diameters (1.5 and 0.7 mm.) connected to a vacuum pump, the larger tube being used to keep the operative field dry, and the smaller tube to suck out the pituitary itself. Surgical asepsis was not essential.

Operation.†† The animal was put on a mouse board, in the horizontal dorsal position, the head proximal to the operator. A mentosternal incision was made, the salivary glands were separated from each other at their mid-line junction and retracted laterally by means of the fine forceps held one in each hand. The left omohyoideus muscle was raised (fig. 1) and divided at the level of the thyroid gland. The blunt hooks were now worked in the plane of cleavage, which was limited by the carotid artery laterally, the thyroid, trachea and pharynx medially and the arch of the superior thyroid artery cranially (fig. 2). In this way access was

* A short description of this technic has been given in Acta brevia Neerlandica, Vol. VII, No. 6/7, 1937.

† From the Laboratory of the Antoni van Leeuwenhoekhuis, Netherlands Institute for Cancer Research, Amsterdam, Holland, and the University of Ghent, Belgium.

†† The illustrations of the successive steps of the operation have been prepared from dissected specimens.

easily gained to the two powerful longus capitis muscles. (A linear narrow cleft between them, filled with loose fatty tissue fig. 2, marks the mid-line.) The blunt hooks, carefully inserted in the medial cleft, brought to light the occipito-sphenoid crest. Careful systematic caudocranial blunt preparation exposed a large surface of the skull-base. The antero-posterior diamcter of the surface extended from the

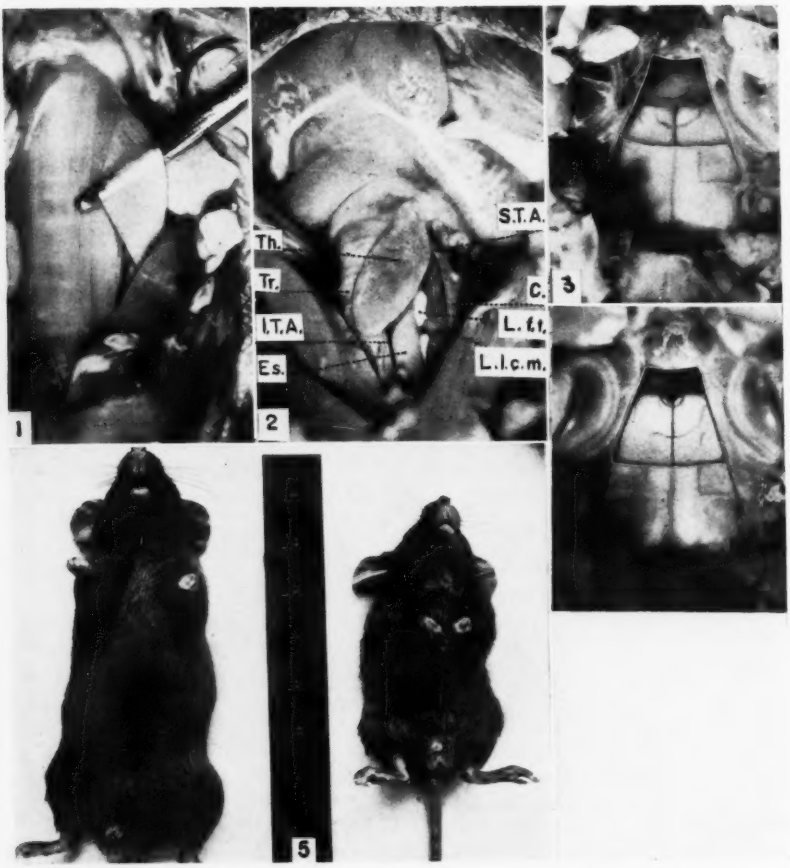

Fig. 1-5. *Th.*, thyroid; *Tr.*, trachea; *I.T.A.*, inferior thyroid artery; *Es.*, esophagus; *S.T.A.*, superior thyroid artery; *C.*, carotid; *L.l.t.*, loose fatty tissue; *L.l.c.m.*, left longus capitis muscle.

proximity of the foramen occipitale to the occipito-sphenoidal synchondrosis, and the lateral diameter was the whole breadth of the occipital bone.

The animal was now rotated 180° and put in the Trendelenburg position. With the aid of the smaller of the sharp hooks, a transverse linear breach was made in the occipital bone at the level of the posterior end of the occipito-sphenoidal crest. For greater convenience we broke the occipito-sphenoidal crest first, an easier grip being obtained on the bone in this way. Great care had to be taken at this moment not to injure the brain, which is here in close contact with the skull. When the

linear breach had been made, the sharp hook was brought farther forward. Its point was introduced into the cartilage of the synchondrosis, and a lateral movement impelled to the instrument so as to split open the synchondrosis. A more or less rectangular panel of bone was now isolated in the base of the skull. When the synchondrosis was completely loose, the larger hook was inserted into it, the panel jerked out of its position, and removed with the aid of the small forceps. The whole pituitary and part of the base of the brain, including the basilar artery and its branches, were thus laid bare (fig. 3). The pituitary was sucked out from its capsule without injuring the diaphragma sellae, under direct visual control and by means of the small (0.5 mm.) suction tube (fig. 4), precisely as in hypophysectomy in the rat. The skin incision was closed with a continuous suture.

It was necessary to interrupt the operation from time to time to let the animal breathe freely. The operation presents no difficulty in young animals not exceeding 20 gm. in weight, and having a thin skull and a broad, soft synchondrosis. It is possible in mice of any size. However, we have not been able to keep alive lactating young afterwards. Figure 5 shows one of our youngest animals 38 days after operation, which occurred on the day it was old enough to leave the mother (20 days). Its weight at the time of operation was 9 gm.; its weight, when photographed, 8 gm. A mouse of the same litter, shown as a control, weighed 22 gm.

The whole procedure may sometimes be performed in as short a time as 4 minutes; one can reckon on an average routine speed of 5 to 6 operations in an hour. With mice above 20 gm., the operation becomes more difficult, and with advancing age in the mouse the difficulties increase because, (a) the bone is thicker and has a tendency to bleed; (b) the synchondrosis is tough and narrow and can only be split open with great effort; a slight injury to the adjacent bone, accompanied by troublesome haemorrhage, would be inevitable.

Postoperative mortality. We agree with Selye, Collip and Thomson that the postoperative mortality is somewhat lower in mice than in rats. If the animals are kept at a temperature of 80° and amply provided with food and drink, the operative mortality in mice not exceeding 20 gm. is insignificant. As many as 30 mice have been operated on in a series without one casualty. The early postoperative mortality (i.e., during the first 48 hours) seems to differ between one variety of mouse and another. It was almost nonexistent among the mice on which most of our work has been done (genetic constitution: F_1 daughters and sons of an albino mother: strain 0 gen. 20 and a dilute brown Murray-Little strain father), and which show practically the same vitality within a few hours following the operation as before its performance. On the contrary, in other varieties of mice, it may reach 20 per cent. The later death rate also seems to differ between one variety of mouse and another, being low among the above-mentioned crosses. This is apparently attributable to endocrine insufficiency. The mortality rate cannot yet be stated in numerical terms, because the first operative successes date back only to the middle of April, 1937, and mice of widely different varieties and ages were operated on.

In mice weighing more than 20 gm. at the time of operation, the early mortality rises rapidly with increasing age, apparently due to haemorrhage combined with removal of the gland. It can be reduced by performing the hypophysectomy in 2 stages, at intervals of 24 or 48 hr. In the first stage, the brain and pituitary are exposed, and the wound is closed; in the second stage the wound is re-opened and the pituitary removed. An interval of over 48 hr. is not practicable, because the gap in the base of the skull will have become occupied by new connective tissue. The later death rate in these animals is comparatively high.

DISCUSSION

The technic described above presents the advantages of simplicity, speed, total exposure of the pituitary and its removal under direct vision, the advantage of an operation performed at an early age (animals weighing as little as 9 gm. being within range of the procedure), with a low mortality when young animals are used§. The one disadvantage with mice weighing over 20 gm. is the difficulty of the operation and its dangers, which increase with the age of the mouse.

I wish to express my obligation to Dr. R. Korteweg, who provided me with every possible facility for this experimental study, as well as advice and sustained encouragement, and my sincere thanks to Mr. Pittock of University College, London, for the excellent illustrations he has prepared.

§ An incidental advantage of this technic, apart from hypophysectomy, is the direct access it affords to the base of the brain.

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DOES ESTRONE RAISE THE METABOLIC RATE?*

J. F. McCLENDON

Following the report by Laqueur, Hart and de Jongh[1] that estrone increased the B.M.R. of ovariectomized mice, McClendon and Burr[2] reported a slight rise in the

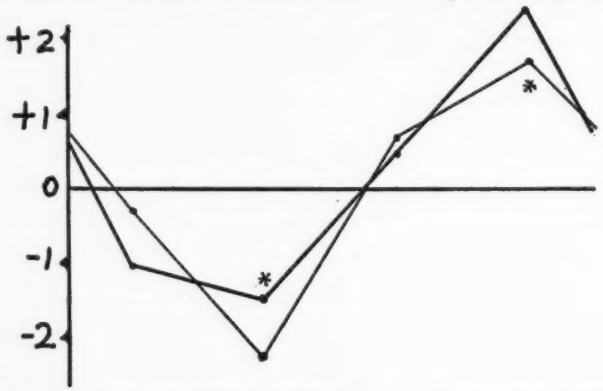

Fig. 1. The percentage rise (+) or fall (—) of the B.M.R. averaged for each week of the menstrual cycle. The ordinate at the left represents the beginning of the first day of menstruation, and the 4 weeks of the cycle extend to the right. The heavy line connects the weekly averages made by Claire Conklin. The stars represent the averages of the postmenstrual and premenstrual weeks of the measurements made by Lillas Myrick, and the light line connects the weekly averages of the measurements made by Rubenstein.

B.M.R. of a woman with dystrophia adiposogenitalis, on the injection of 1000 or more M.U. of estrone. After the injection of 30,000 M.U. of estrone in oil into another woman with dystrophia adiposogenitalis, there was a rise in B.M.R. that continued into the second day. Following the report by Frank and Goldberger and by Hirsch, it was shown by Wildebush and McClendon[3] that the estrone of the blood was lowest during the second week of the menstrual cycle and increased

* From the Laboratory of Physiological Chemistry, University of Minnesota Medical School, Minneapolis, Minn.

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again during the third week. Therefore, in 1930, Conklin and McClendon[4] studied the B.M.R. during the menstrual cycle and obtained average values, shown in the heavy curve in figure 1. Professor Lillas Myrick repeated this work, using the volume of the nipple as an index of the amount of estrone in the blood, and correlating it with the B.M.R. The probable errors were worked out by McClendon, Myrick, Conklin and Wilson,[5] and two points on the curve are shown by the stars on figure 1. The increase in B.M.R. from post to premenstrual week was only 2.53 per cent, but the increase in the nipple volume during the same period was 12.8 per cent.

The change in B.M.R. during the menstrual cycle was studied by Rubenstein,[6] and if these results are averaged by weeks there is a curve such as is represented by the light line in figure 1. Although Rubenstein does not refer to our work, the curves indicate that the Rubenstein data confirm those of Conklin and Myrick. The fact that the B.M.R. is influenced by many factors besides the estrone content of the blood makes it necessary to average a large number of measurements. Myrick's averages are based on 342 measurements and Conklin's on 438, on individuals selected for regular breathing rhythm.

Recently Collett, Smith and Wertenberger[7] observed an increase in the B.M.R., due to administration of estrone to overiectomized women. The interrelation of gonads, hypophysis and thyroid makes this subject very complicated, but Gomez and Turner[8] have shown that the hypophysis is not necessary for nipple growth.

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[4] Conklin, C. J., and J. F. McClendon: Arch. Int. Med. 45:125. 1930.
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THE CARE AND HANDLING OF THE EUROPEAN BITTERLING AS AN EXPERIMENTAL ANIMAL IN ENDOCRINE RESEARCH

ABNER I. WEISMAN AND CHARLES M. ROTHBURD*

In June, 1936 the Southern Medical Journal editorially described the bitterling test for male hormones as proposed by Kleiner, Weisman and Mishkind[1] and concluded as follows: "The simplicity of the technic should make the method of great value if confirmed." This test is based upon the elongation of an egg-laying tube, the ovipositor, which normally is extruded only during the breeding season, but which can be stimulated to lengthen at will by the simple addition of urine containing its natural hormones. Whether the ovipositor lengthening is a response to male hormones and substances of similar structural formulae, as suggested by Kleiner et al[2,3,4] or a response to the female hormone, as originally stated by Fleischmann and Kann,[5] or a response to the cortical adrenal hormone, as reported by Barnes et al[6] is not certain. Corroboration is lacking in each of these findings, due to death of the fish because of inexperience in their care.[7] The handling of the fish is a problem which in itself requires much intensive study, hence confirmation of the work of Kleiner et al has not been forthcoming. Findings based on reactions of sick fish are as worthless as results obtained when using unhealthy capons or rats.

The following facts were gathered together after 3 years of intensive work with the European female bitterling in its experimental environment, in order to encourage and stimulate interest in the endocrine aspects of bitterling research.†

Transportation of the fish. The bitterlings are obtainable through the facilities of the New York Aquarium. In taking the fish from the Aquarium, it is advisable that they be transported in large wide-mouth gallon-size thermos jugs. The fish are shipped in the same water in which they have been living at the Aquarium. They should not be shaken much, and they should arrive at their destination in a few hours. (For distances outside of Greater New York, special arrangements can be made with the Aquarium for shipping and care.)

Water and temperature. The water problem is the most important phase of fish research. When the fish arrive at their destination they should be emptied into a tank containing an amount of water equal to that in which they arrive. The water should have been previously drawn from the tap of running cold water, and allowed to stand at room temperature for a few days prior to the arrival of the bitterlings. If the fish come in water the temperature of which is lower

* From the Endocrine Division of the Gynecology Service of Dr. Edward Hoenig, Jewish Memorial Hospital, New York, N.Y.

† We express our gratitude and appreciation to Dr. C. M. Breder, Jr., acting director of the New York Aquarium, and to Mr. C. W. Coates, also of the New York Aquarium, for their continued assistance and advice regarding the life habits of the European bitterling, both in wild life and as an aquarium specimen. We also thank the New York Zoological Society for offering to us such splendid opportunities, through the Aquarium, to study fish and their relation to medicine.

[1] Editorial Notes in South M. J. Vol. 29, No. 6, 1936.
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than that of room temperature, it should be elevated by the gradual addition of tap water heated for the occasion. Heated water from the hot-water boiler is often harmful to the fish. If the temperature at which the fish are living is higher than that at which they are going to live, equal amounts of both waters can be mixed. The European bitterling is a fish of the temperate zones and is accustomed to varying temperatures; however, it is more adaptable to colder than to warmer temperatures. Hence, the fish fare better in the laboratory under experimental conditions at running water from 71-6° C. and in tanks with a balanced aquarium at from 18-22° C. (room temperatures). Temperatures in excess of 25° C. may be detrimental.

There are a few simple rules to keep in mind. Never place fish in fresh water taken from the tap unless they have previously lived in that same water and at that temperature. Every water supply is different. Each time more water is added to the fish it should have stood in the room for 24 to 48 hours, and should be near the temperature of the stock tank or somewhat colder. The fish live in most types of water except in those which have been heavily treated with chemicals, and each water offers its individual problem. In New York City a thorough testing of various types of water was made expressly for us with the co-operation of Dr. Frank E. Hale, Director of the Laboratories of the Department of Water Supply. In summarizing the results of the water analyses, it was found that the bitterlings could survive in waters containing one-fifth the average amount of free oxygen and 4 times the normal content of carbon dioxide, besides excesses of nitrogenous wastes found in fish tanks. Contrary to the belief that fresh-water fish could not live in water with so little O_2 and so much CO_2, the bitterlings thrived excellently. The pH is evidently not a factor in mortality, since very little change in hydrogen ion concentration was found in any of the waters in and about New York. Moreover, pH concentrations which were distinctly acid and alkali were tested on the fish with no mortality.

Over-population. The ratio of 2 fish to a gallon of water has been found to be a satisfactory living condition in a tank of non-flowing water. In tanks of running water, many times this number can be safely kept. Death of the fish in many instances has followed the introduction of excessive numbers into tanks insufficiently large. The weakest of the fish die first and commence to putrefy and spoil the water. This is soon followed by death of the remainder of the fish. The tanks used should have a large surface area in comparison to their total cubical content. The more shallow and larger the surface area of the tank the better for the fish.

Oxygen and carbon dioxide. In a tank of running water there need be no concern over the amounts of carbon dioxide and oxygen. The local water supply authorities pay particular attention to aeration and filtration of water and deliver a potable water which in most instances is suitable. If the tanks are set up with no running water, oxygen should be added artificially after a time, either by air-pumps, oxygen-donating plants or from time to time changing the water in part. The carbon dioxide has to be removed by the plants or by removing appreciable amounts of the stagnant water. Sunlight, however, is essential for plant physiology. If the aquaria do not receive sunlight, the plants serve no use but rather decompose and are in fact harmful. Where there is insufficient sunshine artificial light from an ordinary tungsten electric light light bulb will serve as a substitute. Green algae are also desirable in the water, although large amounts finally obscure the fish in the summer-time when there is an excess of sunlight.

Nourishment. The fish do best on live worms, and the common Tuberfex

variety are most suitable. The bitterlings have now been living on artificially prepared foods such as Paclum and Dryco (ordinary baby foods). These are almost complete foods. The fish are fed once daily. Over-feeding of artificial foods is dangerous, since not only does the individual fish over-eat but the excess of food settles at the bottom of the tank and causes early putrefaction. The debris in the form of feces and undigested putrefying food is removed from the bottom of the tank by suction with a piece of bent glass tubing. When live food is used, the water in the fish tanks does not have to be changed as often as when dried foods are used. The use of common snails and a catfish or two serve as excellent scavengers, and help remove much of the debris. The water in a well-balanced aquarium has scarcely to be touched.

Signs of toxicity. The bitterlings are so sensitive to toxic substances that they react almost at once when subjected to toxic causes. Signs of distress are characteristic. The fish immediately rise to the surface of the water, with their mouths extending above the surface. Their bodies hang with tail-end lowered, and they swim about sluggishly and aimlessly. Food is not sought for, or noticed, and they respond slowly to light, tactile and other stimuli. They do not stay beneath the water for more than a few seconds before they rise again to the surface. Soon after the toxic cause is removed, or they are removed from the toxic water, the fish return to the lower part of the tank, swim about freely, commence to eat food, and respond quickly to external stimuli.

Death from external causes. Fumes from escaping gas, ether, chloroform and especially formalin found in the hospital laboratory are toxic to the fish. The room in which the fish are kept should be well ventilated and have plenty of sunlight. Water which is heavily treated with copper sulphate is harmful to the fish. Faulty plumbing and corrosion of old pipes may also account for death of the bitterlings.

Solubility of hormones in water. Kurzrok[8] found no reaction in the bitterlings to male hormones, to which other investigators ascribe high biologic stimulation of the ovipositor. Again it must be stressed that fish differ from other laboratory animals. Unless the substances tested upon the bitterlings be soluble or miscible in water in some form, the results obtained with these substances must be disregarded. The male hormones are insoluble in water. Investigations with these crystalline hormones on the bitterlings must be performed with the use of propylene glycol or some other solvent, as suggested and used by Kleiner et al[9] in their experiments, if the work is to have any value. Hence, unless Kurzrok recognizes these general chemical considerations his cursory investigation must be considered valueless.

Lengthening of the ovipositor. Undue agitation of the water, or excitement as in transportation, will cause spontaneous elongation of the ovipositor. Excessive heat and high room temperatures will make the fish ill, and at times cause a spontaneous elongation. During the summer months, while in the breeding season the fish are apparently refractile. In the healthy state and when handled correctly the bitterlings respond only to direct hormone stimulation.

Summary. The European female bitterling test, a fish test for the presence of hormones in normal urines, is explained. Suggestions are made for the care and handling of the fish in order to encourage further work and corroboration of the female bitterling test.

[8] Kurzrok, R.: The Endocrines in Obstetrics and Gynecology, Williams & Wilkins, Baltimore, 1937.
[9] Kleiner, I. S., A. I. Weisman and D. I. Mishkind: Science 85:75. 1937.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright	Murray B. Gordon	A. E. Meyer
Wayne J. Atwell	Arthur Grollman	Warren O. Nelson
Geoffrey Bourne	Frank A. Hartman	J. P. Pratt
Israel Bram	E. C. Hamblen	A. T. Rasmussen
John C. Burch	F. C. Koch	Elmer L. Sevringhaus
H. J. Deuel, Jr.	Edward Larson	L. B. Shpiner
John C. Donaldson	J. T. Lewis	D. L. Thomson
Maurice H. Friedman	Joseph M. Looney	R. L. Zwemer

ADVANCE ABSTRACTS

Partial inhibition of sex activity in the intact female rat by injected estrin. Ball, Josephine. To appear in this Journal.

The normal, sharply cyclic changes in sexual receptivity were prevented in 8 intact female rats by injecting estrin for 2 months in changing doses which varied from 0.25 R.U. to 5 R.U. daily. Behavior during the injection period showed a fairly constant low degree of sexual receptivity, rated 0 to 3 on a scale of 12, whereas the untreated heat periods before and after estrin administration were characterized by peak ratings of 7 to 12. It is concluded that estrin given in small daily doses is, in a sense, a sex depressant for the intact female rat.

Endocrine factors influencing tumor development. The effect of prolan upon the Marsh-Buffalo adenocarcinoma. Bischoff, F., and M. Louisa Long. To appear in this Journal.

In a series of 61 virgin female mice of the Marsh-Buffalo strain, monthly treatment with prolan to produce 200 to 300% ovarian hypertrophy, and therefore marked hyperovarian function, failed to influence the onset or incidence of the adenocarcinoma of the breast to which this strain is subject and which is normally enhanced in breeding animals. The first mammary tumor developed at the age of 9 months. By the 21st month of age, 76% of the controls had developed adenocarcinoma of the breast, 13% had developed lymphosarcoma, and 11% had died of causes not related to tumor formation. Of the treated mice, 65% had developed adenocarcinoma of the breast, 16% had developed lymphosarcoma, and 13% had died of causes not related to tumor formation. It is concluded that the endogenous production of estrin and its related compounds by the ovary through prolan stimulation does not influence carcinogenesis, and that the production of carcinogenesis by massive doses of estrin (exogenous) may be without physiologic significance.

Diabetic traits in a strain of rats. Cole, Versa V., and B. K. Harned. To appear in this Journal.

Seventy % of the glucose tolerance curves of sexually mature, male rats of the Woman's Medical College colony, Yale strain, are diabetic in type. Animals of the Wistar strain give only normal curves. Diabetic glucose tolerance curves do not appear in rats of the Yale strain under 50 days of age, and the average tolerance curve for these animals is identical with that of the Wistar strain of the same age group. In the Yale age groups 51 to 60 days and 61 to 70 days, 14% of the animals in each group give diabetic curves; in the group 71 to 90 days of age 50% of the animals give diabetic curves; and after 90 days of age 70% of the tolerance curves are diabetic. The probable error for the tolerance curves of the Yale strain averages 4 times that of the Wistar strain. The average normal tolerance curve of adult Yale rats parallels reasonably well the curve of adult Wistar rats, but at each point exceeds the Wistar average by approximately 30 mg. %. The fasting (15-16 hr.) true blood sugar of sexually mature rats of the Yale strain exceeds the Wistar average by approximately 30 mg. %. The diabetic trait appears to be inherited.

The effect of gonadotropic hormones on the persisting corpora lutea in hypophysectomized rats. Greep, R. O. To appear in this Journal.

Adult female rats were treated with luteinizing preparations for 5 days prior to hypophysectomy. The ovaries lost 45% of their weight in the first 5 postoperative days; 52% by 15 days and 57% by 40 days. The initial drop was due to follicular involution; the corpora lutea persisted, however, and later comprised the great bulk of the ovaries along with a few primordial follicles. All experimental treatments were started on the 15th day after hypophysectomy. Pituitary FSH stimulated follicular growth, and maintained continuous vaginal estrus. The matured follicles underwent atresia without becoming luteinized, and were replaced. The persisting lutein bodies were not affected. Pituitary LH produced no follicular growth and no estrus, but the corpora lutea were destroyed. With simultaneous FSH and LH therapy the growth of a first crop of follicles and the involution of the persisting corpora proceeded independently. These follicles became luteinized on the 4th to the 6th day, following which another crop of follicles arose and the newly formed corpora regressed. After about the 13th day these cyclic processes became irratic and the gonads underwent profound regression, due presumably to exhaustion of the follicular apparatus and destruction of the last formed corpora lutea. The vaginal smears showed periods of estrus which coincided with periods of follicular dominance.

Injections of estrogen-free PU extract produced long-continued estrus in the absence of any follicular growth. Damaging the persisting corpora lutea with LH previous to the urine injections prevented the appearance of estrus. The evidence indicated that persisting corpora lutea secrete estrin when stimulated with pregnancy urine. The persisting corpora lutea proved to be non-functional, and attempts to stimulate them to produce the progestational hormone have thus far been unsuccessful.

Cellular changes in the anterior hypophyses of vitamin-A deficient rats. Sutton, T. S., and B. J. Brief. To appear in this Journal.

A significant increase was found in the 'basophilic' cells in the hypophyses of vitamin-A deficient rate of both sexes. The increase in basophil count was greatest in the males. The results indicate a partial castration effect on the hypophysis in vitamin-A deficiency.

Histologic effects induced in the anterior pituitary of the rat by prolonged injection of estrin with particular reference to the production of pituitary adenomata. Wolfe, J. M., and A. W. Wright To appear in this Journal.

The pituitaries of 37 rats receiving injections of estrin for periods of from 30 to 425 days were studied. In rats treated for the shorter periods a markedly uniform hypertrophy of the gland was induced; in animals injected for prolonged periods the gland was not only markedly increased in weight but presented localized areas of congestion and swelling, and in some instances gross abnormalities in shape. In rats treated for shorter periods of time the histologic reaction was generalized. There was a marked degranulation of the chromophilic cells accompanied by hypertrophy of the Golgi apparatus. The chromophobes were markedly increased in size and number. In rats injected for prolonged periods the generalized histologic reaction was replaced by one characterized by the appearance of adenomatous foci of cells. Three types of adenomata were found. These included chromophobe adenomata associated with marked vacuolar changes, small nodular foci of cells presenting structural characteristics differentiating them from the surrounding tissue, and diffuse adenomatous hyperplasia of the entire gland. The appearance of small clear vacuoles in many of the cells leading to degeneration is described.

ADRENALS

The effect of adrenal cortical extract upon the oxygen consumption of normal human beings. Hitchcock, F. A., R. C. Grubbs and F. A. Hartman, Am. J. Physiol. **121**:542. 1938.

Experiments carried out on 12 human subjects by means of the Tissot-Haldane technic showed that the administration of adrenal cortical extract produced no significant effect on the basal oxygen consumption. The oxygen consumption was significantly reduced in 9 of the 12 subjects while standing erect, and in 10 of the 12 subjects while walking on a tread-mill at the rate of 100 meters per minute. This effect does not always parallel the content of vital hormone, and so may be due to some other factor. The effect is cumulative and persistent, and in some cases repeated injections cause a reversal of the effect.—F. A. Hitchcock.

The treatment of Addison's disease. Hoagland, R. J., South, M. J. **30**:1208. 1937.

This article covers briefly our present knowledge of treatment. Cortical extracts have proved expensive, difficult of administration, and so far unreliable in their action. The symptoms of Addison's disease are associated with a disturbance of the sodium potassium ratio in the blood. Sodium chloride, together with sodium carbonate or citrate, should be used to maintain the sodium level, whereas the potassium intake should be kept as low as possible. The details of a case treated on these principles are given.—J. C. D.

Adrenal secretion produced by K (Acción adrenalinosecretora del potasio). Hug, E., Rev. Soc. argent de biol. **13**:483. 1937.

Eighteen experiments were made on chloralosed dogs with Tournade and Chabrol's suprarenojugular transfusion. Intraortic injection of KCl (1-5 mg. per kg.) produced adrenal discharge. Adding Ca so as to maintain the normal Ca:K ratio suppressed the adreno-secretory effect of K. Adrenalin, when injected 2-5 minutes before the K, and large doses (8 mg. per kg.) of yohimbin also suppressed the effect. Complete denervation of the adrenals performed 10 days previously did not suppress the adreno-secretory effect of K.—J. T. L.

Comparative action of K and adrenalin on arterial blood pressure (Acción comparada del potasio y de la adrenalina sobre la presión arterial). Hug, E., Rev. Soc. argent. de biol. **13**:476. 1937.

Twenty-nine experiments were made in dogs anesthetized with chloralose with both vagi cut. Intravenous injection of 1-5 mg. per kg. of K (as KCl) increased the blood pressure by a peripheral (vascoconstrictor) effect. There was no quantitative relationship between the hypertensor effects of K and adrenalin. Yohimbin (sympathico- and adrenolytic drug) reversed the effect of adrenalin on the blood pressure, but did not modify that of K. Cocain and pyrogallol increased the effect of adrenalin without modifying that of K. The effect of K sometimes added itself to that of adrenalin, there was no sensitizing to adrenalin by K injection. Ca added to K so as to maintain the normal Ca: K ratio suppressed the K effect.—J. T. L.

The rôle of vitamins B_2, B_4 and B_6 in the absence of the adrenals (Die Rolle von Vitamin B_2, B_4 und B_6 bei Nebennierenmangel). Laszt, L., and F. Verzár, Arch. f. d. ges. Physiol. **239**:134. 1937.

Seven doubly adrenalectomized rats, weighing 40 to 50 gm., were maintained in normal growth for 1 to 2 months when their diet was supplemented with a crude extract of yeast. The addition of vitamins B_1, B_4, B_6 or lactoflavin (B_2) was ineffective. Flavinphosphoric acid was, therefore, considered to be responsible for the observed effectiveness of the yeast extracts.—A.G.

Effect of eserin on adrenal secretion (Acción de la eserina sobre la secreción de adrenalina). Lewis, J. T., and F. N. Gallo, Rec. Soc. argent. de biol. **13**:489. 1937.

Experiments in chloralosed dogs were made by means of Tournade and Chabrol's technic of adreno-jugular transfusion. Eserin sulphate in large doses (intraaortic injection) produced adrenal secretion by direct action on the gland. It sensitized the gland to the adreno-secretory effect of splanchnic stimulation and a quaternary ammonium (p-oxiphenyl-ethyltrimethylammonium); this last even 2 months after complete denervation of the gland. Peripheral stimulation of the vagus did not produce adrenal secretion. In eserinized dogs peripheral stimulation of the vagus frequently produced a slight discharge of adrenalin.—J. T. L.

The effects of various gonadotropic substances upon the ovaries, pituitaries and adrenals of animals receiving long-term injections of estrin. Diaz, J. T., Doris Phelps, E. T. Ellison and J. C. Burch, Am. J. Physiol. **121**:794. 1938.

Mature female rats were injected daily with estradiol benzoate for 60-70 days; some of the animals then received simultaneous injections of gonadotropic hormone for 5-10 days, and the ovaries, pituitaries and adrenals of these animals finally removed and examined. The estrous cycles of some of these long-term treated animals were also examined. The ovaries of the estrin-treated animals showed a marked decrease in size, with a general regression of germinal and interstitial elements. These atrophic changes were all absent in those animals simultaneously treated with gonadotropic substance. No qualitative differences in the effects of various gonadotropic preparations could be detected. The gonadotropic hormone treatment also changed the vaginal smears from the cornified cell type to one in which a mixture of leucocytes and

mediated epithelial cells predominated. The estrin injections caused marked pituitary hypertrophy, but no significant change in the adrenals. The addition of gonadotropic hormone usually caused further hypertrophy of the pituitary and slight hypertrophy of the adrenals. The authors believe this evidence indicates that the estrin acts directly on the pituitary, causing, after long periods of injection, an exhaustion of the gonadotropic hormone production by the gland. Atrophy, but not damage, of the ovary then occurs owing to inadequate stimulation.—S. Cohen.

Arrhenoblastoma. A malignant ovarian tumor associated with endocrinological effects. Norris, E. H., Am. J. Cancer **32**:1. 1938.

A case of arrhenoblastoma with clinical, laboratory and postmortem findings is reported. This is apparently the first in which careful study of all the endocrine glands has been made. It constitutes the 38th of which record was found but only 29 are regarded as authentic. From a review of the literature and detailed study of the present case the author concludes that there is no justification for regarding arrhenoblastoma as arising from any particular part of the primitive sex gland. An essential criterion of the disorder is a condition of intersexuality to which it gives rise. The manifestations include both defeminization and demasculinization manifested by such conditions as amenorrhoea; sterility; atrophy of the breasts, endometrium and genitalia; excessive hair growth; hyper-pigmentation; coarsening of the features; lowering of the pitch of the voice; hypertrophy of the clitoris; masculine body contours; abnormal deposits of fat; and changes of the psyche. In the case described there was degranulation and hyalinization of the basophilic cells of the anterior pituitary, closely similar to those characteristic of Cushing's syndrome. Marked hypertrophy of both the acidophilic and pale cells of the adrenal cortex was evident. The thyroid showed 'extreme quiescence'. No thymus could be found. In spite of the clinical presence of hyperglycemia and glycosuria in such cases, the islands of Langerhans were normal, as were the parathyroids and the pineal gland.—R. G. H.

Homeoplastic transplantation of adrenal glands in rats of inbred strains. Ingle, D. J., G. M. Higgins and H. W. Nilson, Am. J. Physiol. **121**:650. 1938.

Homeografts of the adrenal gland were made within two closely inbred strains of rats, and between animals of these two inbred strains. Of the 56 animals grafted with adrenals taken from donors of the same strain, 48 survived for 120 days and showed regeneration of the grafted cortical tissue, whereas 8 died of adrenal insufficiency soon after grafting and showed completely degenerated grafts. Of the 26 animals grafted with adrenals taken from donors of the other strain 25 died of adrenal insufficiency and showed degenerated grafts. One lived 120 days, but also showed degenerated grafts and was found to have accessory adrenal tissue. The authors conclude that close similarity in the genetic constitution of donor and host is essential for the regeneration and function of homeografts of the adrenal gland.—C. Duncan.

The response of the plasma potassium level in man to the administration of epinephrin. Keys, A., Am. J. Physiol. **121**:325. 1938.

Six normal fasting men were used as subjects. Blood samples were taken before and after the intravenous injection or epinephrin (.005-.3 mg.). A series of samples, collected over a period of 50 minutes after the injection, was studied. Na, K, total base, Cl, cell volume and hemoglobin were determined. Contrary to the previous finding in dogs, cats and rabbits in which the injection of epinephrin caused an immediate marked rise in plasma potassium, injection of epinephrin in man caused a fall in the plasma potassium level. Normal or nearly normal levels were observed in about 20 minutes, and a supra-normal value was generally observed after about 40-60 minutes. These changes were accompanied by an immediate sharp rise in red cell concentration and a somewhat smaller rise in plasma Na. Little or no change in total base was observed.—Lena Lewis.

Renal function in normal and adrenalectomized opossums and effects of post-pituitary and cortico-adrenal extracts. Silvette, H., and S. W. Britton, Am. J. Physiol. **121**:528. 1938.

The effect of adrenalectomy on the renal function of the opossum was studied by following the daily urinary Na, Cl, water and urea excretion. Observations were made on normal and adrenalectomized animals following the administration of 1% or 0.1% NaCl solution by mouth, the injection of post-pituitary solution and cortico-adrenal extracts. The adrenalectomized opossum on 1% NaCl showed a decreased ability to eliminate salt and water. Administration of postpituitary solution to such animals restored the salt and water

excretion to normal or above normal levels. (No antidiuretic effect was observed in either the normal or adrenalectomized opossum.) The urea excretion was essentially normal. On the contrary, adrenalectomized opposums when given 0.1% NaCl excreted more than the normal amount of salt and water, and administration of post-pituitary extract produced a further increase. Cortico-adrenal hormone injections caused a favorable reaction through a marked reduction in Na and Cl excretion, the untreated animal losing 100% more than the treated.—Lena Lewis.

The effects of adrenal cortical extract in rest and work. Missiuro, V., D. B. Dill and H. T. Edwards, Am J. Physiol. **121**:549. 1938.

The subcutaneous injection of adrenal cortical extract produced no significant effects in resting subjects, and did not change the capacity for anaerobic work. Following the administration of the extract, the efficiency with which easy walking was performed was increased for some days, the blood pressure in the early stages of recovery was lower and the resting values reached sooner.—F. A. Hitchcock.

The effect of adrenalectomy upon blood phospholipids and total fatty acids in the cat. Yeakel, Eleanor H., and E. W. Blanchard, J. Biol. Chem. **123**:31. 1938.

The fatty acid and phospholipid content of the whole blood and plasma of 13 adrenalectomized cats was determined by Boyd's method. There was a decrease in the lipids of the whole blood, plasma and cells. There was an increase in the ratio of lipids between cells and plasma during insufficiency. This change is thought to be due to an accumulation of the lipids in the tissues.—J. M. L.

ENDOCRINE GENERAL

Effect of sympathin on blood sugar. Bodo, R. C., and A. E. Benaglia, Am. J. Physiol. **121**:728. 1938.

Cats under chloralose or chloralosane anesthesia were used, the anesthetics being chosen because they did not influence blood sugar. The adrenal medulla of both sides was inactivated and the sympathetic nerves of the liver were destroyed. Sympathin, liberated by stimulation of the cardio-accelerator nerves, raised blood sugar. The production of sympathin was independently checked by the reaction of the nictitating membrane. The rise of blood sugar was greatest (100-200 mg. %) if the postganglionic sympathetic fibers of the liver had been cut a few days previously, probably because of a resulting sensitization of the liver for sympathin. The hypoglycemia became noticeable only about an hour after nerve stimulation and lasted for several hours. The effect of sympathin agrees in this respect with that of adrenalin.—E. Bolzler.

Hyperglycemia produced by sympathin in emotional excitement. Bodo, R. C. and A. E. Benaglia, Am. J. Physiol. **121**:738. 1938.

Adrenal-inactivated and liver-denervated cats without anesthesia were used. Emotional excitement with or without struggle (produced by barking dog and tying on board respectively) caused only a slight rise of blood sugar, whereas a marked hyperglycemia was observed in normal cats. The small changes obtained are considered to be due to sympathin, and were absent in completely sympathectomized animals.—E. Bozler.

Nor-epinephrine [ß-(3, 4-dihydroxyphenyl)-ß-hydroxyethylamine] as a possible mediator in the sympathetic division of the autonomic nervous system. Greer, C. M., J. O. Pinkston, J. H. Baxter, Jr. and E. S. Brannon, J. Pharmacol. & Exper. Therap. **62**:189. 1938.

From observations on the vasomotor system, the nictating membrane, uterus, iris and intestine, the authors conclude that the responses produced by dl-nor-epinephrine and 'liver sympathin" are strikingly similar. The authors suggest that 2 'adrenergic' mediators may be liberated, acting either locally or diffusing into the blood stream to affect distant structures. Of these mediators, the l-nor-epinephrine has a greater power of increasing contraction but a lesser action in producing relaxation than l-epinephrine.—E. L.

Demonstration of a humoral mechanism in hypertension of renal origin (Demostración del mecanismo humoral de la hipertensión nefrogena). Houssay, B. A., and J. C. Fasciolo, Bol. Acad. nac. de med. Buenos Aires P. 342. 1937.

Chloralosed and recently nephrectomized dogs were grafted with a kidney taken from a dog with high blood pressure produced by partial occlusion of the renal artery; in 5-10 minutes

the blood pressure of the receptor had increased 32-70 mm. Hg. Removal of the grafted kidney produced a gradual drop in the blood pressure. Kidneys from normal dogs with normal blood pressure did not produce hypertension when grafted into normal dogs.—J. T. L.

The influence of denervation of the liver on carbohydrate metabolism (Effetti della enervazione del fegato sul metabolismo degli idrati di carbonio). Ljvraga, P., Arch. per le sc. med. **64**:411. 1937.

Denervation of the liver at the hilus brings the blood sugar to the lowest level that normally occurs and lowers also the glucemic curve in the glucose test. The effect wears off within three months. It causes a drop in blood sugar in dogs with mild diabetes but is not efficacious after total removal of the pancreas. Adrenalin has the same effect before and after the operation. The method is inapplicable in the treatment of human diabetes.—A. E. Meyer.

Treatment of the menopause syndrome by irradiation of the pituitary gland. Zollinger, R., and W. W. Vaughan, New England J. Med. **217**:219. 1937.

Irradiation of the pituitary in dosages of 1600 r. in 14 patients suffering from hot flashes gave improvement in only 35% of the cases. Sham treatments given to 3 patients produced no change in the symptoms. There was no change in the B.M.R., which was low both before and after treatment, in the majority of the patients. There was no change in the sugar-tolerance curves, and no change in the vaginal biopsies before and after treatment.—J. M. L.

Enterocrinin, a hormone which excites the glands of the small intestine. Nasset, E. S., Am. J. Physiol. **121**:481. 1938.

The name enterocrinin (intestinal secretagogue) has been proposed for a hormone which causes an increased secretion by the intestinal glands. The method for testing preparations on dogs permitted simultaneous assay of vasodilatin, secretin and enterocrinin. Crude preparations made by extracting intestines with 0.03 to 0.05 normal HCl in 85 to 87% alcohol almost always contained all 3 substances. By further purification, products which affected only intestinal secretion (at low dosages) were obtained. Results with one preparation indicated the threshold dose to be 6 to 8 γ per kg. of body weight, which was considerably below the dose necessary to affect blood pressure or the pancreas. The preparations increased the amount of enzymes as well as fluid in the secretions. Studies on the distribution of enterocrinin in the animal body indicated its presence only in the small intestine and colon. Secretin preparations contained enterocrinin. Some chemical properties have been given.—H. W. Schultz.

HYPOPHYSIS

Electrophoresis of posterior pituitary gland preparations. du Vigneaud, V., G. W. Irving, Jr., Helen M. Dyer and R. R. Sealock, J. Biol. Chem. **123**:45. 1938.

A preparation of posterior pituitary gland containing 5,610 U of pressor and 3090 U of oxytocic material was placed in a 19-cell electrophoretic apparatus and allowed to migrate for 17 days under a potential of 14,000 volts. The pressor principle accumulated in the cathode cell 6 times as fast as the oxytocic principle. No loss of activity occurred up to 6 days, and then a gradual destruction of both fractions took place. The results indicate that the two principles are different chemical entities. Separation of the active fractions can be carried out by means of this technic.—J. M. L.

The possible hypophysary pathogeny of vitiligo and other disturbances of human pigmentation (La posible patogenia hipofisaria del vitiligo y de otros trastornos de la pigmentación humana). Marañón, G., C. Richet, M. Sourdel and H. Netter, Semana méd. **45** (Part 1):113. 1938.

The melanophore hormone of the pituitary probably influences the physiological regulation of pigmentation in humans as in other mammals and in amphibia. Certain disturbances of pigmentation are concomitant with signs of pituitary dysfunction. Certain other symptoms of that kind are associated with definite conditions of the genital organs, such as pregnancy or lesions of the ovaries. It is suggested that in such cases the pituitary is affected also. A number of cases is reported of leukoderma associated with pituitary symptoms.—A. E. Meyer.

Roentgen irradiation of the hypophysis. Lawrence, J. H., W. O. Nelson and H. Wilson, Radiology **29**:454. 1937.

After irradiation of the pituitary regions of female albino rats 30 to 40 days old there is a temporary decrease in the rate of growth. Histologic examination of the pituitary glands

consistently reveals characteristic changes which presumably account for the retardation of growth, the inhibition of ovarian activity, and the atrophic condition of the thyroid and adrenal glands. From these observations it is fair to conclude that although the pituitary glands of young albino rats are relatively radioresistant, nevertheless they can be influenced by x-ray. The immediate period of suppression is followed by a gradual recovery of the pituitary and, consequently, of the organs influenced by its hormones. No definite clinical implications can be drawn but these experiments suggest that it is not unlikely that the non-adenomatous human hypophysis can be influenced with therapeutic doses of x-rays.—Author's Abst.

Effect of anterior pituitary extract on blood fats (Acción del extracto de lóbula anterior de hipófisis sobre las grasas de la sangre). Muñoz, J. M., Rev. Soc. argent. di biol. 13:321. 1937.

Alcaline (diabetogenic) anterior pituitary extract increased considerably the phospholipids and cholesterol. This increase was not produced by similar extracts of other tissues; thyroidectomy, pancreatectomy, castration, removal of the adrenal medulla, the lumbar sympathetic chain and section of the splanchnic nerves did not prevent this effect of the extract. The increase in blood fat was not necessarily dependent on the increase in blood sugar, also produced by the extract. An enormous increase in blood lipids was produced by the extract in pancreatectomized dogs; insulin antagonized this effect of the extract. Phosphorization of lipids mobilized by the extract was less when the pancreas was removed.—J. T. L.

Thyroid and sexual stimulating function of the hypophysis in rats (Tiroides y poder éxcitosexual de la hipófisis en la rata). Reforzo, M. J., Rev. Soc. argent. de biol. 13:399. 1937.

Female infantile rats were used as tests of the sexual-stimulating potency of implanted adult male rat hypophysis. The weight and histological aspect of the ovary were recorded, also the opening of the vagina, occurrence of estrus, and weight of uterus. The ovarian weight of untreated controls was 27.6 mg., with typical infantile aspect; of those implanted with hypophysis from normal rats 74.3 mg., follicular ripening in all cases, luteinization in 50%; with hypophysis from thyroidectomized males (50-80 days) 50.6 mg., follicular ripening in 6-10, no luteinization; with hypophysis from thyroidectomized males fed on thyroid 73.2 mg., follicular ripening and luteinization occasionally; with hypophysis from normal rats fed on thyroid, 90.4 mg., follicular ripening, intense luteinization; with hypophysis from castrated males 118 mg., follicular ripening, intense luteinization; with hypophysis from castrated thyroidectomized 105 mg., follicular ripening and luteinization; thyroidectomized treated with folliculin 40.3 mg., infantile aspect. Thyroidectomy reduced significantly the sexual-stimulating potency of the hypophysis, thyroid feeding almost re-established this potency. Treatment with folliculin reduced even more the potency of the hypophysis. Castration considerably increased the potency of the hypophysis; whereas simultaneous thyroidectomy did not reduce this increase significantly. Thyroid feeding increased the potency of the hypophysis of normal rats, especially the luteinizing effect.—J. T. L.

Ketonemia produced by anterior pituitary extract in dogs (Elevación cetonémica por extracto anterohipofisario en el perro). Rietti, C. T., Rev. Soc. argent. de biol. 13:312. 1937.

Ketonemia in normal dogs amounted to 2 mg. % (expressed in acetylsalycilic acid); in hypophysectomized dogs it was 1.86 mg. Anterior pituitary extract produced an increase in ketonemia in normal and thyroidectomized dogs, also after removal of the adrenal medulla and section of the splanchnic nerves. In pancreatectomized dogs, the already high ketonemia was increased even more by the extract; insulin had an antagonistic effect. Extracts similarly prepared from other tissues did not produce these effects.—J. T. L.

GONADS

Effect of prolonged treatment with large doses of oestrone on the endocrine and sexual organs of the rat (Acción de las grandes dósis prolongadas de estrona sobre los órganos endócrinos y sexuales en la rata). Del Castillo, B. E., and R. Sammartino, Rev. Soc. argent. de biol. 13:455. 1937.

Normal and castrate (12 days after operation) male rats (7 animals) and female (6 animals) rats were given 2400 U estrodiol benzoate subcutaneously twice weekly, the treatment lasting 250-300 days. The weight and histological aspect of the organs were determined at the end of the experiment. The hypophysis was considerably increased in weight, chromophobe cells

predominated, basophile cells were decreased or absent, eosinophile cells vacuolized and decreased. The thyroid had lost weight, the colloid was homogenous and stained lightly, the cells were cubic and vacuolized; a direct relationship between these alterations and the weight of the hypophysis was found. The parathyroids showed groups of vacuolized cells. The adrenals were increased in weight, in 3 cases adenomatous nuclei were found, and the fasciculated zone showed increase in vaculolae. Blood sugar was low in cases with large hypophysis. Blood pressure was normal. Testes and ovaries were considerably atrophied. The uterus was increased in weight, the mucosa had a stratified epithelium with vacuolized cells, the chorion became infiltrated by eosinophile cells, and the myometrium hypertrophied. The uterine os showed muscular hypertrophy; there was cornification of the epithelium, invading the uterine horns below the uterine epithelium. The fallopian tubes showed muscular hypertrophy, stratification and vacuolization of the epithelium. The vagina had a thickened and cornified epithelium. The seminal vesicles had a low epithelium, fibrosis and hyalinization of the muscular coat.—J. T. L.

Activation of testosterone by higher fatty acids and their sodium salts. Ehrenstein, M., and E. L. Corey, J. Biol. Chem. **122:**297. 1938.

The effect of the simultaneous injection of palmitic or stearic acids, or their acid salts, with testosterone was determined by the weight of the seminal vesicles and prostate of castrated male rats. The materials were injected in sesame oil for 9 days and the animals sacrificed on the 10th day. Both free acids and salts caused an increase in the action of the testosterone. This effect was thought to be due to the constant slow liberation of the hormone.—J. M. L.

The occurrence and action of adrenalin in ovaries (Über Vorkommen under Wirkung von Adrenalin in Ovarien). Euler, U. S. v., and S. Hammarström, Skandinav. Arch. f. physiol. **77:**163. 1937.

The presence of epinephrine in extracts of cow or pig ovaries was demonstrated by pharmacological and colorimetric methods. From 1 to 2 γ of epinephrine were found in each gram of fresh tissue. The chromaphil cells, which are demonstrable in the ovarian stroma, are probably responsible for the formation of this epinephrine. Epinephrine constricts the ovarian blood vessels.—A. G.

Investigations concerning temporary, physiological sterility of woman (Untersuchungen zur Frage der temporaren, physiologischen Sterilität der Frau). Guthmann, H., and H. Vetter, Monatschr. f. Geburtsh u. Gynäk. **104:**129. 1937.

A study of the menstrual and sexual histories of 145 women indicated that the time of greatest fertility varied with the time interval of the menstrual cycles. In 22 to 25-day cycles, conception occurred from the 6th to the 12th day; in the 26 to 29-day cycle, conception occurred from the 8th to the 17th day; in the 30 to 33-day cycle, conception occurred from the 9th to the 24th day. It was concluded that conception was possible in the post and premenstruum, although this was not likely. The data indicated a short period of viability for the ovum and sperm. Physiologic sterility cannot be accepted as a substitute for operative or radiologic sterilization, where this is indicated for medical or eugenic reasons.—M. R. White.

Morphologic investigations of the inhibiting action of the follicle hormone on the mammary glands of lactating rats (Morphologische Untersuchungen über die hemmende Wirkung des Follikelhormons auf die Brustdrüse laktierender Ratten). Herold, L., Zentralbl. f. Gynäk. **62:**155. 1938.

The lactating rats were treated with 10 daily doses of from 500 to 3000 I.U. of commercial estrins, which showed increasing suppression of lactation with increasing dose, consequent undernutrition of the young, and the histological sections show that the inhibition lasts even well beyond the cessation of treatment. The alveoli are first empty, and then atrophy toward the resting breast stage.—E. L. S.

The problem of pregnancy, hydatid mole, chorionepithelioma, and Aschheim-Zondek reaction (Zum Problem Schwangerschaft, Blasenmole, Chorionepitheliom und Aschheim-Zondek-Reaktion [AZR]). Isbruch, F., Zentralbl. f. Gynäk. **62:**161. 1938.

A case report is given of a 27-year old woman, curetted for an early incomplete abortion, at which time tissues suggested a mole. The A-Z test soon became negative, menses returned, but stopped after 3 flows. The A-Z test became very strongly positive, but the patient was correctly believed to be pregnant, and went to term. Pelvic findings in early pregnancy were

reassuring, and later the APL titre decreased. High titre cannot be assumed as being diagnostic of mole or chorionepithelioma.—E.L.S.

Use of the gonadotropic hormone of pregnancy urine in the treatment of male sexual underdevelopment. Lawrence, C. H., and A. M. Harrison, New England J. Med. **217**:89. 1937.

A study of the action of gonadotropic hormone from pregnancy urine was made in 122 published cases of cryptorchidism. In unilateral cases the mechanical factors are more important and the endocrine elements less so than in bilateral cases or hypoplastic testes. The treatment was successful in 50% of the unilateral group, 72% of the bilateral group and 73% of the eunuchoid. It is advisable to give at least 600 R.U. weekly and even 1000 to 2000 R.U. weekly to more resistant individuals. Best results were obtained in cases in which treatment was started well before the age of puberty. Allergic reactions to pituitary extracts sometimes occur and an intradermal test should be made in all cases in which the history is suggestive.—J. M. L.

Limitation of milk secretion by follicular hormone (Ueber Hemmung der Milchsekretion durch Follikelhormon). Lindemann, W., Zentralbl. f. Gynäk. **62**:159. 1938.

Three women were promptly relieved of undesired lactation by use of 3 different commercial estrins. The doses are not clear, although 260,000 I.U. sufficed in one case. A new theoretical explanation is offered without evidence to support it.—E. L. S.

Factors influencing the augmentation effects produced by zinc or copper when mixed with gonadotropic extracts. Bischoff, F., Am. J. Physiol. **121**:765. 1938.

The influence of pH and site of injection on the augmentation effects produced by zinc and copper salts on gonadotropic extract was studied. Using immature rats, the increased ovary weights produced by the salt-hormone mixtures were compared. Zinc salts were found to be less effective than copper salts in the salt-hormone mixtures adjusted to an acid pH (pH 5.0), but were nearly as effective at a more alkaline pH (pH 8.5). Both salts precipitated the hormone from solution at pH 8.5, but not at pH 5.0. When injected separately and at a site removed from the hormone injection site, neither salt produced an augmentation effect. The site of injection of salt-hormone mixture was found to be an important factor in the degree of augmentation produced. The greatest effect was produced by subcutaneous injection, somewhat less by intradermal, and practically no effect by intraperitoneal injection. The author believes that the augmenting action of the salts is due to the production of divided dosage or slow absorption. This could be caused by the presence of an insoluble form of the hormone (at pH 8.5) and/or (at pH 5.0) by the trapping of the hormone due to precipitation, with subsequent necrosis of the tissue constituents at the site of injection. The high augmenting powers of copper salts at an acid pH is explained by the fact that copper salts produce more local necrosis than zinc salts.—S. Cohen.

Suppression of lactation by follicular hormone (Supresión de la secreción láctea por medio de la hormona folicular). Brea, A. M., and C. C. Sontag Gándara, Semana méd. **44**:1463. 1937.

In 27 cases of established lactation, treatment was begun with 10,000 U of dehydrofolliculine benzoate daily. Three patients did not respond, 2 of them even after increase of the dosage. Two patients responded to increased doses; in the remainder, lactation promptly ceased and concomitantly mammary pain disappeared.—A. E. Meyer.

Results with the Visscher-Bowman pregnancy test. Dunn, R. D., and F. J. Northway, Am. J. Obst. & Gynec. **35**:298. 1938.

On 395 urine samples the test was found accurate in 84.8% of known pregnancies, 87.6% of suspected pregnancies, and 54.8% of non-pregnancies. The test is not dependable.—E. L. S.

Effects of androgenic sterols in hypophysectomized and in castrated rats. Cutuly, E., D. R. McCullagh and Elizabeth Cutuly, Am. J. Physiol. **121**:786. 1938.

The effects of prolonged treatment with dehydroandrosterone acetate, androstenediol, testosterone oxime, testosterone oxime propionate and testosterone propionate, on the changes which occur in the sex organs of the rat after hypophysectomy and/or castration were observed. The organs studied were weighed and examined histologically. In the testes of hypophysectomized rats, injected with these substances, varying degrees of tubular maintenance were induced, but in no instance did such treatment prevent atrophy of the interstitial cells. In both these, and in hormone-treated castrated animals, varying degrees of scrotal flushing and turges-

cence were also noted. The seminal vesicles and prostate in hypophysectomized and in castrated rats were maintained to different extents by the androgenic sterols employed. The authors believe that the testes in hypoyhysectomized rats in some way augment the effect in the accessory sexual organs. Some of the androgenic substances prevented increase in basophiles in the anterior hypophyses of castrated rats, and in some cases also induced basophilic degranulation; no effect was observed on the eosinophiles. When the hormone treatment of the hypophysectomized animals was delayed, the resulting involution of the testes was not repaired, nor could sustained scrotal stimulation be effected; they did, however, bring about repair of the prostate and seminal vesicles.—S. Cohen.

Skin tests for pregnancy. Frank, C. W., and P. B. Wahrsinger, Am. J. Obst. & Gynec. **35**:303. 1938.

Antuitrin-S gave only 68% correct positive tests and many false negatives. Progestin gave almost uniformly positive tests in pregnant and non-pregnant cases.—E. L. S.

Antuitrin-S cutaneous test for the diagnosis of pregnancy. Gersch, I., Am. J. Obst. & Gynec. **35**:301. 1938.

Using antuitrin-S and their own placental gonadotropic extract, as well as phenolized saline, the false negatives found were 20.8%, and men showed false positives in 81.6%, non-pregnant women in 66.6%.—E. L. S.

The red cell count of macaques in relation to the menstrual cycle. Gutkelch, A. N., and S. Zuckerman, J. Physiol. **91**:269. 1937.

The red blood cell count, urine output and in some cases water balance studies were followed in about 20 monkeys (Macacca nemestrina and mulatta). Both normal cycles and estrin-injected castrates were followed. Hemoconcentration as followed by the red blood count was highest in the middle of the cycle. Estrin injection also gave an increase in the count, in one case from 4¾ to 5¾ million cells. The results are discussed in relation to those found in species exhibiting swelling of the sexual skin, and the hemoconcentration was discovered to occur in other species as well.—R. L. Zwemer.

Effects of castration and of sexual hormones on the adrenals of male rats. Hall, K., and V. Kovenschensky, J. Physiol. **91**:365-374. 1938.

This is an histological study of the adrenals of 53 normal and 153 castrated rats. Most of the latter were injected with various androgens. The castration adrenal was hypertrophied, with an increase in fasciculata and reticularis zones, increased vacuolation and disappearance of a 'demarcation zone' due to lipoid increase. Androgens restored a normal appearance or, if given in overdose, still further depleted lipoids. The castrated, treated, depleted adrenal returned to normal 9 days after cessation of treatment. Testosterone propionate also reduced the lipoids of normal rat adrenal glands.—R. L. Zwemer.

The isolation of estrogenic diols from the urine of pregnant mares. Hirschmann, H., and O. Wintersteiner, J. Biol. Chem. **122**:303. 1938.

From 20,000 liters of pregnant-mare urine 9.88 gm. of crude phenolic estrogens were obtained by extraction with ether and adsorption on magnesium oxide from a dioxane-ether solution. This material was separated into ketonic and non-ketonic fractions by means of betaine hydrazide (Girard's reagent). The non-ketonic phenols were purified and fractionated between ether and 2% NaOH. The alkaline extract yielded a diol, 17- dihydro-equilenin. The ether extract yielded β-estradiol. The physiological potencies of these compounds were much less than those of the corresponding ketones. A third component of the composition $C_{18}H_{22}O_2$, which was thought to be an unsaturated diol, was also obtained in small amounts. The use of spectrographic analysis was found to be indispensable for controlling the course of fractionation.—J. M. L.

Do androgens affect the blood lipids? Kochakian, C. D., P. L. MacLachlan and H. D. McEwen, J. Biol. Chem. **122**:433. 1938.

The injections of androstenedione into a thin castrate, a fat castrate, and a normal dog had no immediate or delayed effect on the blood plasma lipid values, cholesterol, phospholipid, neutral fat, or total lipid. Testosterone oxime and testosterone benzoate likewise failed to exert any effects on the level of the plasma lipids of castrate dogs. No correlation was found between the duration of castration or diet on the level of the blood plasma lipids.—J. M. L.

The occurrence of female sex hormone in muds used for therapeutic baths (Beitrag zum Vorkommen der Weiblichen Sexualhormone in Moor- und Schlam-marten). Kilian, V., Klin. Wchnschr. **16**:1316. 1937.

Tests of the benzol extracts made from dried muds used in the therapeutic baths in Czechoslovakia revealed by the Allen-Doisy test method a hormone content of 500-600 R.U. per kilogram.—M. H. F.

Water metabolism in relation to the menstrual cycle. Krohn, P. L., and S. Zuckerman, J. Physiol. **88**:369. 1937.

Water balance studies were made during 3 cycles of a sexually mature female, pig-tailed. The amount of water retained during the phase of sexual swelling agreed closely with the observed increase in body weight. Urine excretion was much greater during subsidence of the sexual skin swelling.—R. L. Zwemer.

Gonadotropic hormone therapy in cryptorchidism and disturbances of spermatogenesis. McCahey, J. F., Pennsylvania M. J. **41**:359. 1938.

Prolan, the gonadotropic principle found in the urine of pregnant women, has been found useful in certain affections of the male genitals. Although the injection of prolan into immature female rats brings about maturation-like changes in the ovarian follicles, similar injections into immature males do not induce premature spermatogenesis. In both young and adult male rats, these injections stimulate the endocrine function of the testes, which results in marked enlargement of the accessory sex organs. However, Collip (1931) has shown that this testis endocrine response is limited to a period of about 60 days, after which the enlarged accessory organs regress to about normal size, even though the injections are continued. Smith and Leonard (1934) found that in hypophysectomized rats the period of testis endocrine response to the injection of prolan was only about 20 days, but during this time the testicular atrophy which invariably results from hypophysectomy was checked, and the animals remained potent and fertile. A maximum of 1000 R.U. of prolan is sufficient to cause descent of the testes in the majority of cases of cryptorchidism, which are benefited by this form of therapy. In instances in which complete descent is not secured with this quantity, additional injections will be useless. One exception to this general rule should be mentioned. When the testes are abdominal in position, it is advisable to continue the injections, as good results have been obtained in such cases after 2000 R.U. have been given. In the occasional case of deficient spermatogenesis in association with subthyroidism the use of proper dóses of thyroid substance may result in some improvement.—I. B.

The chemical pregnancy test of Visscher and Bowman. Messinger, W. J., M. H. Presberg, and M. D. Fellows, Am. J. Obst. & Gynec. **35**:295. 1938.

Trial of the method in 187 cases showed accuracy of 69.5%, with more false negatives than positives. The authors have eliminated APL, histidine, creatine, glucose, lactose, and galactose as the responsible agents for the test.—E. L. S.

The presence of estrogenic hormones in maternal and fetal circulation. Soule, S. D., Am. J. Obst. & Gynec. **35**:309. 1938.

Maternal and fetal (cord) blood samples were taken at the same time, and the Fluhmann test made, using dilutions of 1:2 to 1:20. This showed uniformly the same concentration of estrin in both types of blood. It is concluded that the placenta is permeable to estrin.—E. L. S.

Tissue loss from the endometrium during menstruation. Watson, M. C., and E. W. McHenry, Am. J. Obst. & Gynec. **35**:316. 1938.

Two monkeys were treated with estrone and progestin in sequence, and sections from the uteri taken 1 and 5 days after therapy. These are shown to demonstrate that there is marked tissue degeneration and dissolution leading to tissue loss preceding the menstrual flow. Their theory is that the accumulation of materials for secretion makes return to the resting stage impossible when the stimuli to secretion are withdrawn.—E. L. S.

PANCREAS

Some effects of zinc and iron salts on the hypoglycemic action of insulin in rats. Blatherwick, N. R., Mary E. Ewing and Phoebe J. Bradshaw, Am. J. Physiol. **121**:44, 1938.

Investigation on rats of the effects on hypoglycemia, produced by adding salts of zinc and

iron to solutions of insulin, showed that these are dependent upon factors such as the concentration of the metal, whether or not the metal is in solution, and the ratio of metal to insulin.—Authors' Summary.

Pancreatic endocrine function in phloridcin diabetes (La función del páncreas en la diabetes floridzínica). Houssay, B. A., and V. G. Foglia, Rev. Soc. argent. de biol. **13**:370.

Dogs received 1 gm. phloridzin in 20 cc. olive oil subcutaneously daily for 4 days. The pancreas was then removed and grafted into dogs made diabetic through pancreatectomy performed the previous day. The blood sugar of the recipients fell to normal (100 mg.%) in 4 hr., when the grafted pancreas came from a normal dog or one injected with olive oil (6 exp.). When it came from a phloridzinized dog (8 experiments) this occurred in only 1 case. In the other cases, the blood sugar showed a slight decrease (5 experiments), it remained at the initial level, or it continued rising. Intravenous injection of 1 gm. glucose per kg. into phloridzinized dogs, nephrectomized the day before giving the glucose, produced a more prolonged hyperglycemia (4 experiments) than in the controls; 2 U insulin per kg. given simultaneously with the glucose re-established the normal blood sugar curve in phloridzinized dogs (4 experiments). The response to insulin is normal in phloridzinized dogs. Diabetic (pancreatectomized) dogs, on receiving phloridcin, showed an increase (2 experiments) in glycosuria, a drop in blood sugar to hypoglycemic level, and coma, not relieved by glucose injection. Subtotally pancreatectomized dogs (3 experiments) after phloridzin injection had an increase in glycosuria and hypoglycemia. Hypoglycemia in these experiments is due to renal elimination of sugar, since pancreatectomy and nephrectomy in phloridzinized dogs was followed by a considerable hyperglycemia (2 experiments). Muscle glycogen increased slightly (less than in controls) in phloridzinized dogs (3 experiments) 1 hr. after intravenous injection of 2 gm. glucose per kg. It is concluded that insulin secretion is diminished but not completely suppressed in phloridzin diabetes.—J. T. L.

The present mortality of diabetic children—a remediable and therefore hopeful index of the future of the diabetic child. Joslin, E. P., Ann. Int. Med. **11**:1348. 1938.

A study of the causes of 104 deaths in a series of 1071 cases of diabetes in children shows that, although coma was the most common cause in the first 5-year period following the advent of insulin, it has decreased during each succeeding 5-year period. During the past 5 years it was the cause in 43% of the deaths, which is slightly higher than for all ages in the 8-year period before insulin. Tuberculosis and other infections were found to increase as causes of death during each of the 3 to 5-year periods.—R. R. Durant.

The effects of insulin hypoglycemia upon the diabetic heart in children and youth. Root, H. F., Ann. Int. Med. **11**:1332. 1938.

Electrocardiographic studies of the effects of insulin hypoglycemia on the heart were made on 25 young subjects, 20 of whom were between 7 and 15 years of age. The changes noted were slight and not correlated with the variations in blood sugar.—R. R. Durant.

Protamine insulin in the treatment of juvenile diabetes. White, Priscilla, South. M. J. **31**:15. 1938.

During the last 2 years 604 juventile patients have been adjusted to the protamine zinc insulin. Four of these have died and can not be traced; 17 have discontinued it, and 574 are using it. These patients were adjusted so that, with a single daily dose, they were about 90% sugar free. By using this figure instead of 100%, severe reactions were avoided. Regular insulin was used in conjunction with the protamine insulin, if that was found necessary. The effect on the health and development of the patients has been better than with ordinary insulin, and fewer of the complications have been observed. Charts and careful analysis of methods and results cover the subject in detail.—J. C. D.

PARATHYROID

Post-operative parathyroid tetany. Swinton, N. W., New England J. Med. **217**:165. 1937.

An incidence of 0.2% of postoperative parathyroid tetany was reported in a series of 13,000 thyroid operations. The prevention of tetanic symptoms requires adequate exposure of the thyroid gland and adjacent structures to avoid injury or removal of the parathyroid glands. A description of the operative technic is given, and a discussion of the symptomatology in the 26 affected cases. A new vitamin D substance, dehydrotachysterol, called 'AT-10,' was the

most satisfactory therapeutic agent to maintain normal Ca and P levels, and to control tetany. —J.M.L.

Parathyroid grafts in experimental hypoparathyroidism. (Hipoparatiroidismo experimental. Biología de los injertos paratiroideos). Rojas, P., and F. J. Manfredi. Rev. Soc. argent. de biol. **13**:390. 1937.

Thyroparathyroidectomized dogs were grafted in the neck muscles or in the kidney, and parathyroid tissue was taken from the same or another dog. Blood Ca decreased and P increased after parathyroidectomy and returned to normal after the grafting. Autotransplants gave a larger percentage of 'takes' than homotransplants; the kidney was a more favorable site for the graft than the neck muscles. In 10 hours vascularization of the periphery of the graft was sufficient to assure the survival of a considerable amount of tissue; the central part degenerated and was invaded by connective tissue, which later became vascularized. After 20 days the graft was found stabilized. At 90 days the glandular tissue started to grow into the central zone, previously degenerated. At 4 months the grafts showed the structure of a normal gland. Removal of the grafts produced tetany, decrease in blood Ca and increase in P.—J. T. L.

PITUITARY

Evaluation of the skin tests for pregnancy. Graffaguino, P., and E. von Haam, South. M. J. **31**:169. 1938.

Among the tests examined was one using antuitrin-S. An erythema at the site of injection within half an hour was regarded as a negative reaction. The results were correct in 59% of the cases, wrong in 9%, and doubtful in 31%.—J. C. D.

Anterior pituitary gonadotropic and anterior pituitary-like hormones in the treatment of cryptorchidism. Turner, H. H., South. M. J. **31**:381. 1938.

The author offers a summary of 26 patients with cryptorchidism, who have been treated with APL hormone. Descent of the testes has been obtained in 14 (53.9%), partial success in 8 (30.8%), and failure of descent in 4 (15.3%). The average treatment took 25 weeks, with a total dosage of 13,000 R.U., corresponding to about 500 U weekly. In general, no serious complications were observed, although in one patient precocious sexual development and, in another, softening of the testicle were suggested. Endocrine therapy is of definite value as a preliminary to and following orchidopexy. The increased size of the testicle and lengthening of the cord elements permit better surgery, and the postoperative results are greatly improved by the treatment. Endocine therapy should be given sufficient trial in perhaps every case of undescended testes, and instituted preferably before the 8th year. The treatment is simple, without apparent harm, and with definite benefit to the majority of patients.—J. C. D.

THYROID

Preparation of a purified thyrotropic hormone by chemical precipitation. Lambie, C. G., and V. M. Trikojus, Biochem. J. **31**:843. 1937.

A potent thyrotropic hormone (at least 10 Junkmann-Schoeller U per mg.) was obtained by extracting acetone dried ox anterior pituitary glands with acetic acid (0.5%), precipitating protein with sulpho-salicylic acid (20%) and then the hormone by the addition of sodium tungstate (10%) to the acid liquor. After removal of the tungstic acid (with barium hydroxide) the hormone was reprecipitated with alcoholic benzoic acid and freed from the latter by washing with acetone, and precipitating from its solution in dilute aqueous ammonia with the same solvent. The purified hormone was thus obtained as a white powder, easily soluble in water, but still it gave a ppt. in concentrated solution with sulpho-salicylic acid. It was free from gonadotropic activity and could be readily tolerated by humans on injection. It was almost completely destroyed in solution at 100°C.—G. B.

Iodine response and other factors in their relation to mortality in thyrotoxicosis. Lerman, J., New England J. Med. **217**:1041. 1937.

In a series of 1187 patients with toxic goiter, of whom 731 had adequate metabolic data, it was found that 3.4% failed to respond to iodine. The mortality rate of this group was 24.0%, whereas in the responding cases it was 1.1%. The most important factors in these deaths were thyrotoxic 'crises' with or without pneumonia and cardiac failure. The former prevailed most frequently in responding patients, and the latter in the non-responding ones. Males showed less response to iodine than females and had twice their mortality. Both factors were adversely

affected by age. In the responding group the mortality rate varied directly with the level of metabolism. The mortality was higher in patients with nodular goiter than in those with diffuse goiter, and in patients with heart disease than in those without it. A small group of aged patients, with low basal rates, having nodular goiters and cardiac involvement accounted for most of the deaths. These patients should receive special study and care.—J. M. L.

The rôle of the thyroid gland in the evolution of infections (Le rôle du corps thyroïde dans l'évolution des infections). Marañon, G., Progrès med. P. 1377. Oct. 2, 1937.

The study of a large number of subjects of thyroid disorders leads to the conclusion that thyroid insufficiency increases resistance to general infections. In the Spanish province of Las Hurdes, where endemic cretinism exists in an acute and limited form, and practically the entire population was hypothyroid, 10% of the adults had suffered from measles and scarlet fever in childhood. In neighboring villages, free from cretinism, and with practically the same living conditions prevailing, the people with this incidence averaged 36%. In the sporadic cases in Madrid, these antecedents were positive in 16% of the cases, and in an equal number of non-hypothyroid individuals it was 41%. The problem has been studied particularly in regard to tuberculosis. In Madrid, 66% of the hyperthyroid individuals have mild tubercular lesions. In 100 sporadic hypothyroid cases there were only 7 with tubercular lesions. In hypothyroid cases with latent tubercular lesions, thyroid treatment may hasten the evolution of tuberculosis. In Las Hurdes, in 1922, tubercular lesions could be found in only 5% of the inhabitants, in spite of unhygienic conditions. Fourteen years after launching a vigorous campaign against endemic cretinism, the latter had subsided considerably, but the number of tubercular patients had trebled.—Author's Abst.

The serum sodium content in myxedema (Der natriumgehalt des Blutserums bei Myxodem). Margitay-Becht, A., Klin. Wchnschr. **16:**1353. 1937.

Serum sodium values were normal in patients with thyrotoxicosis, acromegaly, and pituitary cachexia. In myxedema, however, the values were decreased to the lower limit of the normal range, and the serum proteins were markedly increased. With thyroid medication there was a rise in serum sodium and a fall in serum protein.—M. H. F.

The iodine tolerance test as an aid in the diagnosis of clinical hyperthyroidism. Perkins, H. J., and F. H. Lahey, New England J. Med. **216:**501. 1937.

Blood-iodine determinations were made on 25 normal subjects, 25 with non-toxic adenomatous goiter, 25 with clinical hyperthyroidism and 28 with a questionable hyperthyroidism, after the ingestion of 6 minims of Lugol's solution. The normal group showed an elevation of blood iodine from 7.2 to 132 γ, the goiter group an elevation from 8.2 to 125 γ, and the hyperthyroid group only from 13.1 to 82 γ. An elevation above 100 γ was considered as the dividing line between normal and hyperthyroid cases. In the last group, 18 patients rose above 100 γ, and of these 10 cases gave no evidence of hyperthyroidism. Three subjects were in iodine remission, and 1 was operated upon without improvement. The test, although not specific, is an aid in establishing a diagnosis of hyperthyroidism.—J. M. L.

Studies of chronic morphine poisoning in dogs. VII. Effect of thyroid feeding on the excretion of morphine in tolerant and non-tolerant dogs. Plant, O. H., and D. Slaughter, J. Pharmacol. & Exper. Therap. **62:**106. 1938.

The influence of powdered thyroid, 4 gm. daily for 3 days on the excretion of 3 daily doses of morphine, 20 mg. per kg. was studied in 4 non-tolerant and 3 tolerant dogs. In the non-tolerant group, the average total excretion of morphine for 3 days decreased 12.6%, whereas with the tolerant group there was an increase of 32%. The diuresis produced by thyroid feeding in the tolerant dogs may have increased the urinary excretion of the alkaloid, but diuresis is not the only factor, as fecal excretion also was increased.—E. L.